



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : C07K 5/06, C07C 259/06, C07K 5/08	A1	(11) International Publication Number: WO 00/34313 (43) International Publication Date: 15 June 2000 (15.06.00)
(21) International Application Number: PCT/EP99/09519 (22) International Filing Date: 6 December 1999 (06.12.99) (30) Priority Data: 60/111,661 10 December 1998 (10.12.98) US (71) Applicant: F. HOFFMANN-LA ROCHE AG [CH/CH]; Grenzacherstrasse 124, CH-4070 Basle (CH). (72) Inventors: DANKWARDT, Sharon, Marie; 814 Quetta Avenue, Sunnyvale, CA 94087 (US). VAN WART, Harold, Edgar; 80 Arbuelo Way, Los Altos, CA 94022 (US). WALKER, Keith, Adrian, Murray; 12798 Canario Way, Los Altos Hills, CA 94022 (US). (74) Agent: BRAUN, Axel; 124 Grenzacherstrasse, CH-4070 Basle (CH).		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: PROCOLLAGEN C-PROTEINASE INHIBITORS <div style="text-align: center;"> </div> <p style="text-align: right;">(1)</p>		
(57) Abstract <p>This invention relates to compounds of Formula (I) wherein R¹-R⁷, A, Z and n are as described in the summary of the invention that are inhibitors of procollagen C-proteinase, pharmaceutical compositions containing them, methods for their use and methods for their preparation.</p>		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakhstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

- 1 -

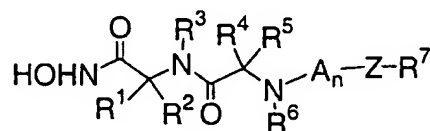
Procollagen C-proteinase Inhibitors

The present invention relates to compounds that inhibit procollagen C-proteinase, pharmaceutical compositions containing them, methods for their use, and
5 methods for preparing these compounds.

The collagens are integral components of connective tissue. At present nineteen types of collagens have been identified. The interstitial collagen types I, II and III are the major collagen components of tissue. These collagens are synthesized
10 as procollagen precursor molecules having amino- and carboxy-terminal peptide extensions also known as pro-regions. These pro-regions are typically cleaved upon secretion of the procollagen molecule to give a mature collagen molecule which is capable of association into highly structured collagen fibers. (see, e.g., Fessler and Fessler, *Annu. Rev. Biochem.* 47, 129, (1978); Kivirikko et al., *Extracellular Matrix Biochemistry* (1984) and Kuhn, *Structure and Function of Collagen Types* (eds
15 Mayne, R and Burgeson, R. E.), Academic Press, Inc., Orlando, Florida pp 1-42 (1987).

Excessive collagen deposition is associated with a variety of fibrotic diseases
20 such as interstitial pulmonary fibrosis, pericentral fibrosis, Symmers' fibrosis, perimuscular fibrosis, kidney fibrosis, endocardial sclerosis, hepatitis, acute respiratory distress syndrome, arthritis, cystic fibrosis, surgical adhesions, tendon surgery, corneal scarring, scleroderma, chronic allograft rejection, hemodialysis shunt fibrosis and restenosis. These diseases are characterized by excessive deposits
25 of fibrillar interstitial collagens that are resistant to proteolytic degradation thus leading to the symptoms of fibrosis. Therefore, inhibition of the pathological deposition of these collagens should help in the treatment of these diseases.

Recent studies suggest that procollagen C-proteinase is the essential enzyme that catalyzes the cleavage of the C-propeptide of types I, II and III collagens and therefore instrumental in the formation of functional collagen fibers ((*see*, Fertala et al., *J. Biol. Chem.*, 269, 11584, (1994)). It would therefore be desirable to provide procollagen C-proteinase inhibitors and thereby provide a means of combating diseases mediated by excessive deposition of these collagens. It is therefore in a first aspect, an object of the present invention to provide hydroxamic acids selected from the group of compounds represented by Formula (I):



(I)

wherein:

R^1 and R^4 are, independently of each other, hydrogen or alkyl;

R^2 is: (i) cycloalkyl, cycloalkylalkyl, aryl, aralkyl, aralkenyl, heteroaryl, heteroaralkyl, heteroaralkenyl, heterocyclo or heterocycloalkyl; or

(ii) -(alkylene)-B-X where B is -O-, -NR⁸-, -S(O)_n- (where n is 0, 1 or 2), -C=O, -CONR⁸-, -NR⁸CO₂-, NR⁸SO₂- or -C(=NR⁸)NR⁸SO₂- (where R⁸ is H or alkyl), and X is cycloalkyl, cycloalkylalkyl, aryl, aralkyl heteroaryl or heteroaralkyl; or

(iii) -(alkylene)-B-X where B is -NR⁸CO- (where R⁸ is H or alkyl), and X is cycloalkyl, cycloalkylalkyl, aryl, aralkyl, heteroaryl or heteroaralkyl; or

(iv) R^2 and R^3 form an alkylene or heteroalkylene chain;

R^3 is hydrogen or alkyl;

R^6 is hydrogen, alkyl, cycloalkyl, cycloalkylalkyl, aryl, aralkyl, heteroaryl or heteroaralkyl;

R^5 is:

(i) hydrogen, alkyl, cycloalkyl, cycloalkylalkyl, aryl, aralkyl, aralkenyl, heteroaryl, heteroaralkyl, heteroaralkenyl, heterocycloalkyl, heteroalkyl, or -(alkylene)-C(O)-X¹ where X¹ is alkyl, hydroxy, alkoxy, aryl, aralkyl, aryloxy, aralkyloxy, heteroaryl, heteroaryloxy, heteroaralkyloxy or NR'R'' (where R' and R'' are independently H or alkyl, or R' and R'' form an alkylene chain); or

(ii) R⁵ and R⁴ form an alkylene chain; or

(iii) R⁵ and R⁶ form an alkylene chain;

n is 0 or 1;

A is -C(=O)-CH(R⁹)-(CH₂)_m-N(R¹⁰)- wherein:

m is an integer from 0-5 inclusive;

R⁹ is hydrogen, alkyl, cycloalkyl, cycloalkylalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl, heterocycloalkyl, heteroalkyl, or -(alkylene)-C(O)-X¹ where X¹ is alkyl, hydroxy, alkoxy, aryl, aralkyl, aryloxy, aralkyloxy, heteroaryl, heteroaryloxy, heteroaralkyloxy or NR'R'' (where R' and R'' are independently H or alkyl, or R' and R'' form an alkylene chain); and

R¹⁰ is hydrogen, alkyl, aralkyl or heteroaralkyl;

Z is Y-B wherein:

Y is alkylene or a bond; and

B is -CO-, -C(O)O-, -CONR⁸-, -SO₂-, or -SO₂NR⁸- (where R⁸ is hydrogen or alkyl), alkylene (optionally substituted by hydroxy, alkoxy, amino, monoalkylamino or dialkylamino) or a bond;

R⁷ is cycloalkyl, cycloalkylalkyl, aryl, aralkyl, heteroaryl or heteroaralkyl;

provided that when n = 0 and Z is SO₂, then R² does not contain an imidazole group; and their pharmaceutically acceptable salts, prodrugs, individual isomers, and mixtures of isomers.

In a second aspect, this invention provides a method of treatment of a disease in a mammal treatable by administration of a procollagen C-proteinase inhibitor selected from the group of compounds represented by Formula (I):

In a third aspect, this invention provides pharmaceutical compositions containing a therapeutically effective amount of a compound of Formula (I) or its pharmaceutically acceptable salt and a pharmaceutically acceptable excipient.

5 In a fourth aspect, this invention provides a method of treating disease by administering to a patient a selective inhibitor of procollagen-C-proteinase.

In a fifth aspect, this invention provides a method of preparing compounds of Formula (I).

10

Unless otherwise stated, the following terms used in the specification and claims have the meanings given below:

"Alkyl" means a linear saturated monovalent hydrocarbon radical of one to
15 six carbon atoms or a branched saturated monovalent hydrocarbon radical of three to six carbon atoms, e.g., methyl, ethyl, propyl, 2-propyl, pentyl, and the like.

"Alkylene" means a linear saturated divalent hydrocarbon radical of one to six
20 carbon atoms or a branched saturated divalent hydrocarbon radical of three to six carbon atoms, e.g., methylene, ethylene, propylene, 2-methylpropylene, pentylene, and the like.

"Heteroalkylene" means an alkylene chain in which one methylene group has
been replaced by O, S or NR' (where R' is hydrogen or alkyl.)

25

"Alkenyl" means a linear monovalent hydrocarbon radical of two to six
carbon atoms or a branched monovalent hydrocarbon radical of three to six carbon
atoms, containing at least one double bond, e.g., ethenyl, propenyl, and the like.

"Alkenylene" means a linear divalent hydrocarbon radical of two to six carbon atoms or a branched monovalent hydrocarbon radical of three to six carbon atoms, containing at least one double bond, e.g., ethenylene, 2-propenylene, and the like.

5

"Acyl" means a radical $-C(O)R$ where R is hydrogen, alkyl, alkenyl, cycloalkyl, cycloalkylalkyl, haloalkyl, aryl, aralkyl, aralkenyl, heteroaryl, heteroaralkyl, heteroaralkenyl, or heterocyclo, e.g., acetyl, benzoyl, thenoyl, and the like.

10

"Acyloxy" means a radical $-OC(O)R$ where R is hydrogen, alkyl, alkenyl, cycloalkyl, cycloalkylalkyl, aryl, aralkyl, aralkenyl, or haloalkyl, e.g., acetoxy, 3,3,3-trifluoroacetoxy and the like.

15

"Acylamino" means a radical $-NRC(O)R'$ where R is hydrogen or alkyl and R' is hydrogen, alkyl, alkenyl, cycloalkyl, cycloalkylalkyl, heteroalkyl, haloalkyl, aryl, aralkyl, aralkenyl, heteroaryl, heteroaralkenyl, or heteroaralkyl, e.g., acetylamino, trifluoroacetylamino, benzoylamino, methylacetylamino, and the like.

20

"Sulfonylamino" means a radical $-NRSO_2R'$ where R is hydrogen or alkyl and R' is alkyl, alkenyl, cycloalkyl, cycloalkylalkyl, heteroalkyl, haloalkyl, amino, monosubstituted amino, disubstituted amino, aryl, aralkyl, aralkenyl, heteroaryl, heteroaralkenyl, or heteroaralkyl, e.g., methylsulfonylamino, benzylsulfonylamino, N-methylaminosulfonylamino, and the like.

25

"Halo" means fluoro, chloro, bromo, or iodo, preferably fluoro and chloro.

"Haloalkyl" means alkyl substituted with one or more same or different halo atoms, e.g., $-CH_2Cl$, $-CF_3$, $-CH_2CF_3$, $-CH_2CCl_3$, and the like.

"Cycloalkyl" means a saturated monovalent cyclic hydrocarbon radical of three to six ring carbons, e.g., cyclopropyl, cyclopentyl, cyclohexyl, and the like.

5 "Carbocycle" means a saturated, cyclic group of 3 to 8 ring atoms in which all the ring atoms are carbon, e.g., cyclopentyl, cyclohexyl, and the like.

"Aryl" means a monovalent monocyclic or bicyclic aromatic hydrocarbon radical of 6 to 10 ring atoms, optionally fused to a carbocycle or heterocycle, and optionally substituted independently with one or more substituents, preferably one or
10 two substituents selected from alkyl, heteroalkyl, haloalkyl, halo, nitro, acyloxy, cyano, cycloalkyl, cycloalkylalkyl, optionally substituted phenyl, optionally substituted phenylalkyl, heteroaryl, heteroaralkyl, -OR (where R is hydrogen, alkyl, haloalkyl, alkenyl, cycloalkyl, cycloalkylalkyl, optionally substituted phenyl, heteroaryl, optionally substituted phenylalkyl, or heteroaralkyl), -NRR' (where R and
15 R' are independently hydrogen, alkyl, alkenyl, haloalkyl, cycloalkyl, cycloalkylalkyl, optionally substituted phenyl, optionally substituted phenylalkyl, optionally substituted phenylalkenyl, heteroaryl, or heteroaralkyl), -C(O)R (where R is hydrogen, alkyl, alkenyl, cycloalkyl, cycloalkylalkyl, haloalkyl, optionally substituted phenyl, optionally substituted phenylalkyl, optionally substituted phenylalkenyl, heteroaryl, heteroaralkyl, or heteroaralkenyl), -S(O)_nR (where n is an integer from 0
20 to 2 and R is hydrogen (provided that n is 0), alkyl, haloalkyl, alkenyl, cycloalkyl, cycloalkylalkyl, optionally substituted phenyl, heteroaryl, optionally substituted phenylalkyl, or heteroaralkyl), -SO₂NRR' (where R and R' are independently hydrogen, alkyl, alkenyl, haloalkyl, cycloalkyl, cycloalkylalkyl, optionally substituted phenyl, optionally substituted phenylalkyl, optionally substituted phenylalkenyl, heteroaryl or heteroaralkyl, or R and R' together with the nitrogen they are attached
25 to form a cycloamino ring), -COOH, -(alkylene)-COOH, -(alkenylene)-COOH, -COOR^a, -(alkenylene)-COOR^a, -(alkylene)-COOR^a (where R^a is alkyl, optionally substituted phenylalkyl, or heteroaralkyl), -CONR' R'', -(alkylene)-CONR' R'',
30 (where R' and R'' are independently selected from hydrogen, alkyl, cycloalkyl,

cycloalkylalkyl, optionally substituted phenyl, optionally substituted phenylalkyl, heteroaryl and heteroaralkyl, or R' and R'' together with the nitrogen they are attached to form a cycloamino ring), -NRC(O)R' (where R is hydrogen or alkyl and R' is hydrogen, alkyl, alkenyl, cycloalkyl, cycloalkylalkyl, haloalkyl, optionally substituted phenyl, optionally substituted phenylalkyl, optionally substituted phenylalkenyl, heteroaryl, heteroaralkenyl, or heteroaralkyl), -NRSO₂R' (where R is hydrogen or alkyl and R' is alkyl, alkenyl, cycloalkyl, cycloalkylalkyl, haloalkyl, optionally substituted phenyl, optionally substituted phenylalkyl, optionally substituted phenylalkenyl, heteroaryl, heteroaralkenyl, or heteroaralkyl), or -NRSO₂NR'R'' (where R is hydrogen or alkyl and R' and R'' are independently hydrogen, alkyl, alkenyl, haloalkyl, cycloalkyl, cycloalkylalkyl, optionally substituted phenyl, optionally substituted phenylalkyl or optionally substituted phenylalkenyl, or R' and R'' together with the nitrogen they are attached to form a cycloamino ring). More specifically the term aryl includes, but is not limited to, phenyl, 1-naphthyl, 2-naphthyl, tetrahydronaphthyl, methylenedioxyphenyl, indanyl, tetralyl, indolyl, chromanyl, isochromanyl and the like.

"Heteroaryl" means a monovalent monocyclic or bicyclic aromatic radical of 5 to 10 ring atoms containing one, two, or three ring heteroatoms selected from N, O, or S, the remaining ring atoms being C. The heteroaryl ring is optionally substituted independently with one or more substituents, preferably one or two substituents, selected from alkyl, haloalkyl, halo, nitro, cyano, cycloalkyl, cycloalkylalkyl, optionally substituted phenyl, optionally substituted phenylalkyl, -OR (where R is hydrogen, alkyl, haloalkyl, alkenyl, cycloalkyl, cycloalkylalkyl, optionally substituted phenyl or optionally substituted phenylalkyl), -NRR' (where R and R' are independently hydrogen, alkyl, alkenyl, haloalkyl, cycloalkyl, cycloalkylalkyl, optionally substituted phenyl, optionally substituted phenylalkyl or optionally substituted phenylalkenyl, or R and R'' together with the nitrogen they are attached to form a cycloamino ring), -C(O)R (where R is hydrogen, alkyl, alkenyl, cycloalkyl, cycloalkylalkyl, haloalkyl, optionally substituted phenyl, optionally substituted

phenylalkyl, or optionally substituted phenylalkenyl), $-S(O)_nR$ (where n is an integer from 0 to 2 and R is hydrogen (provided that n is 0), alkyl, haloalkyl, alkenyl, cycloalkyl, cycloalkylalkyl, optionally substituted phenyl, or optionally substituted phenylalkyl), $-SO_2NRR'$ (where R and R' are independently hydrogen, alkyl, alkenyl, haloalkyl, cycloalkyl, cycloalkylalkyl, optionally substituted phenyl, optionally substituted phenylalkyl or optionally substituted phenylalkenyl, or R and R'' together with the nitrogen they are attached to form a cycloamino ring), $-COOH$, $-(alkylene)-COOH$, $-(alkenylene)COOH$, $-COOR^a$, $-(alkenylene)-COOR^a$, $-(alkylene)-COOR^a$ (where R^a is alkyl, or optionally substituted phenylalkyl), $-CONR'R''$, $-(alkylene)-CONR'R''$, (where R' and R'' are independently selected from hydrogen, alkyl, cycloalkyl, cycloalkylalkyl, optionally substituted phenyl and optionally substituted phenylalkyl, or R' and R'' together with the nitrogen they are attached to form a cycloamino ring), $-NRC(O)R'$ (where R is hydrogen or alkyl and R' is hydrogen, alkyl, alkenyl, cycloalkyl, cycloalkylalkyl, haloalkyl, optionally substituted phenyl, optionally substituted phenylalkyl, or optionally substituted phenylalkenyl), $-NRSO_2R'$ (where R is hydrogen or alkyl and R' is alkyl, alkenyl, cycloalkyl, cycloalkylalkyl, haloalkyl, optionally substituted phenyl, optionally substituted phenylalkyl, or optionally substituted phenylalkenyl), $-NRSO_2NR'R''$ (where R is hydrogen or alkyl and R' and R'' are independently hydrogen, alkyl, alkenyl, haloalkyl, cycloalkyl, cycloalkylalkyl, optionally substituted phenyl, optionally substituted phenylalkyl or optionally substituted phenylalkenyl, or R' and R'' together with the nitrogen they are attached to form a cycloamino ring), or an amino protecting group. More specifically the term heteroaryl includes, but is not limited to, furyl, thienyl, pyrroly, pyridyl, purinyl, pyrimidinyl, pyrazolyl, thiazolyl, imidazolyl, thiazolyl, thiadiazolyl, indolyl, azaindolyl, benzofuranyl, benzimidazolyl, benzthiazolyl, quinolinyl, isoquinolinyl, benzotriazolyl, benzopyranyl, and the derivatives thereof.

"Optionally substituted phenyl" means phenyl ring which is optionally substituted with one or more substituents, preferably one or two substituents selected

from alkyl, haloalkyl, halo, nitro, cyano, -NRR' (where R and R' are independently selected from hydrogen and alkyl, or R and R' together with the nitrogen they are attached to form a cycloamino ring), -OR (where R is hydrogen, alkyl or haloalkyl), -COOR^a (where R^a is hydrogen or alkyl) or -CONR'R" (where R' and R" are independently selected from hydrogen and alkyl, or R' and R" together with the nitrogen they are attached to form a cycloamino ring). Representative examples include, but are not limited to, 4-fluorophenyl, 3,4-dibromophenyl, 4-chloro-2,5-dimethylphenyl, 2,4,5-trichlorophenyl, 4-bromo-2-trifluoromethoxyphenyl, 2-chloro-4-trifluoromethyl, 4-tertbutylphenyl, 4-methoxyphenyl, 3-nitrophenyl, and the like.

"Heterocycle" or "Heterocyclo" means a saturated cyclic radical of 3 to 8 ring atoms in which one or two ring atoms are heteroatoms selected from N, O, or S(O)_n (where n is an integer from 0 to 2), the remaining ring atoms being C, where one or two C atoms may optionally be replaced by a carbonyl group. The heterocyclo ring may be optionally substituted independently with one, two, or three substituents selected from alkyl, haloalkyl, cycloalkyl, cycloalkylalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl, halo, cyano, acylamino, amino, monosubstituted amino, disubstituted amino, -OR (where R is hydrogen, alkyl, haloalkyl, alkenyl, cycloalkyl, cycloalkylalkyl, aryl, heteroaryl, aralkyl, or heteroaralkyl), -C(O)R (where R is hydrogen, alkyl, alkenyl, cycloalkyl, cycloalkylalkyl, haloalkyl, aryl, aralkyl, aralkenyl, heteroaryl, heteroaralkyl, or heteroaralkenyl), -S(O)_nR [where n is an integer from 0 to 2 and R is hydrogen (provided that n is 0), alkyl, haloalkyl, alkenyl, cycloalkyl, cycloalkylalkyl, amino, monosubstituted amino, disubstituted amino, aryl, heteroaryl, aralkyl, or heteroaralkyl], -COOH, -(alkylene)-COOH, -COOR^a, -(alkylene)-COOR^a (where R^a is alkyl, heteroalkyl, aralkyl, or heteroaralkyl), -CONR'R", -(alkylene)-CONR'R" (where R' and R" are independently selected from hydrogen, alkyl, cycloalkyl, cycloalkylalkyl, aryl, aralkyl, heteroaryl and heteroaralkyl, or R' and R" together with the nitrogen they are attached to form a cycloamino ring) or an amino protecting group. More specifically the term heterocyclo includes, but is not limited to, tetrahydropyranyl, piperidino, piperazino,

morpholino and thiomorpholino, thiomorpholino-1-oxide, thiomorpholino-1,1-dioxide, and the derivatives thereof.

The term "cycloamino" means a heterocyclo group in which at least one ring
 5 atom is nitrogen. Specific examples include piperidine, piperazine, morpholine, thiamorpholine, thiamorpholine sulfoxide and thiamorpholinesulphone.

"Heteroalkyl" means an alkyl, cycloalkyl, or cycloalkylalkyl radical as
 defined above, carrying a substituent selected from $-NR^aR^b$, $-OR^c$, or $-S(O)_nR^d$,
 10 wherein:

n is an integer from 0 to 2,

R^a is hydrogen, alkyl, haloalkyl, cycloalkyl, cycloalkylalkyl, aryl, aralkyl,
 aralkenyl, heteroaryl, heteroaralkyl, heteroaralkenyl, or acyl;

15 R^b is hydrogen, alkyl, aryl, aralkyl, acyl, $-SO_2R$ (where R is alkyl, haloalkyl, amino, monosubstituted amino or disubstituted amino), $-COOR$ (where R is alkyl, aralkyl, or heteroaralkyl), $-CONR'R''$, $-(alkylene)CONR'R''$ (where R' and R'' are independently selected from hydrogen, alkyl, cycloalkyl, cycloalkylalkyl, aryl, aralkyl, heteroaryl and heteroaralkyl, or R' and R'' together with the nitrogen they are
 20 attached to form a cycloamino ring);

or R^a and R^b together with the nitrogen atom which they are attached to form a cycloamino ring.

R^c is hydrogen, alkyl, haloalkyl, cycloalkyl, cycloalkylalkyl, aryl, aralkyl, aralkenyl, heteroaryl, heteroaralkyl, heteroaralkenyl, acyl, $-CONR'R''$ (where R'
 25 and R'' are independently selected from hydrogen, alkyl, cycloalkyl, cycloalkylalkyl, aryl, aralkyl, heteroaryl and heteroaralkyl, or R' and R'' together with the nitrogen they are attached to form a cycloamino ring).

R^d is hydrogen, alkyl, haloalkyl, cycloalkyl, cycloalkylalkyl, aryl, aralkyl, aralkenyl, heteroaryl, heteroaralkyl, heteroaralkenyl, acyl, -and additionally when $n =$
 30 0, $CONR'R''$ (where R' and R'' are independently selected from hydrogen, alkyl,

cycloalkyl, cycloalkylalkyl, aryl, aralkyl, heteroaryl and heteroaralkyl, or R' and R" together with the nitrogen they are attached to form a cycloamino ring) and when n = 2, NR'R" where R' and R" have the meanings given immediately above.

5 Representative examples of heteroalkyl include, but are not limited to 2-methoxyethyl, benzyloxymethyl, thiophen-2-ylthiomethyl, and the like;

 "Cycloalkylalkyl" means a radical -R^aR^b where R^a is an alkylene group and R^b is a cycloalkyl group as defined above e.g., cyclopropylmethyl, cyclohexylpropyl, 3-cyclohexyl-2-methylpropyl, and the like.

10

 "Aralkyl" means a radical -R^aR^b where R^a is an alkylene group and R^b is an aryl group as defined above e.g., benzyl, phenylethyl, 3-(3-chlorophenyl)-2-methylpentyl, and the like.

15 "Aralkenyl" means a radical -R^aR^b where R^a is an alkenyl group and R^b is an aryl group as defined above e.g., 3-phenyl-2-propenyl, and the like.

 "Heteroaralkyl" means a radical -R^aR^b where R^a is an alkylene group and R^b is a heteroaryl group as defined above e.g., pyridin-3-ylmethyl, 3-(benzofuran-2-yl)propyl, and the like.

20

 "Heteroaralkenyl" means a radical -R^aR^b where R^a is an alkenyl group and R^b is a heteroaryl group as defined above e.g., 3-pyridin-3-ylpropen-2-yl, and the like.

25 "Heterocycloalkyl" means a radical -R^aR^b where R^a is an alkylene group and R^b is a heterocyclo group as defined above e.g., tetrahydropyran-2-ylmethyl, 4-methylpiperazin-1-ylethyl, and the like.

 "Alkoxy", "aryloxy", "heteroaryloxy", "aralkyloxy", or
30 "heteroaralkyloxy" means a radical -OR where R is an alkyl, aryl, heteroaryl, aralkyl,

or heteroaralkyl respectively, as defined above e.g., methoxy, phenoxy, pyridin-2-yloxy, benzyloxy, and the like.

"Optional" or "optionally" means that the subsequently described event or
5 circumstance may but need not occur, and that the description includes instances
where the event or circumstance occurs and instances in which it does not. For
example, "heterocyclo group optionally mono- or di- substituted with an alkyl group"
means that the alkyl may but need not be present, and the description includes
situations where the heterocyclo group is mono- or disubstituted with an alkyl group
10 and situations where the heterocyclo group is not substituted with the alkyl group.

The term "protecting group" refers to a grouping of atoms that when attached
to a reactive group in a molecule masks, reduces or prevents that reactivity.
Examples of protecting groups can be found in T.W. Greene and P.G. Futs,
15 Protective Groups in Organic Chemistry, (Wiley, 2nd ed. 1991) and Harrison and
Harrison *et al.*, Compendium of Synthetic Organic Methods, Vols. 1-8 (John Wiley
and Sons. 1971-1996). Representative amino protecting groups include formyl,
acetyl, trifluoroacetyl, benzyl, benzyloxycarbonyl (CBZ), *tert*-butoxycarbonyl (Boc),
trimethyl silyl (TMS), 2-trimethylsilyl-ethanesulfonyl (SES), trityl and substituted
20 trityl groups, allyloxycarbonyl, 9-fluorenylmethyloxycarbonyl (Fmoc), nitro-
veratryloxycarbonyl (NVOC) and the like. Representative hydroxy protecting groups
include those where the hydroxy group is either acylated or alkylated such as benzyl
and trityl ethers as well as alkyl ethers, tetrahydropyranyl ethers, trialkylsilyl ethers
and allyl ethers.

25

The term "protected hydroxylamine derivative" refers to a modified
hydroxylamine whose nitrogen and/or hydroxyl groups are protected such that the
nitrogen atom may be selectively monoacylated.

Compounds that have the same molecular formula but differ in the nature or sequence of bonding of their atoms or the arrangement of their atoms in space are termed "isomers". Isomers that differ in the arrangement of their atoms in space are termed "stereoisomers".

5

Stereoisomers that are not mirror images of one another are termed "diastereomers" and those that are non-superimposable mirror images of each other are termed "enantiomers". When a compound has an asymmetric center, for example, it is bonded to four different groups, a pair of enantiomers is possible. An enantiomer can be characterized by the absolute configuration of its asymmetric center and is described by the *R*- and *S*-sequencing rules of Cahn and Prelog, or by the manner in which the molecule rotates the plane of polarized light and designated as dextrorotatory or levorotatory (i.e., as (+) or (-)-isomers respectively). A chiral compound can exist as either individual enantiomer or as a mixture thereof. A mixture containing equal proportions of the enantiomers is called a "racemic mixture".

The compounds of this invention may possess one or more asymmetric centers; such compounds can therefore be produced as individual (*R*)- or (*S*)-stereoisomers or as mixtures thereof. For example, if the R^1 and R^2 substituents in a compound of formula (I) are different, then the carbon to which they are attached is an asymmetric center and therefore the compound of formula (I) can exist as an (*R*)- or (*S*)-stereoisomer. Unless indicated otherwise, the description or naming of a particular compound in the specification and claims is intended to include both individual enantiomers and mixtures, racemic or otherwise, thereof. The methods for the determination of stereochemistry and the separation of stereoisomers are well-known in the art (see discussion in Chapter 4 of "Advanced Organic Chemistry", 4th edition J. March, John Wiley and Sons, New York, 1992).

A "pharmaceutically acceptable excipient" means an excipient that is useful in preparing a pharmaceutical composition that is generally safe, non-toxic and neither biologically nor otherwise undesirable, and includes an excipient that is acceptable for veterinary use as well as human pharmaceutical use. A "pharmaceutically acceptable excipient" as used in the specification and claims includes both one and more than one such excipient.

A "pharmaceutically acceptable salt" of a compound means a salt that is pharmaceutically acceptable and that possesses the desired pharmacological activity of the parent compound. Such salts include:

(1) acid addition salts, formed with inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like; or formed with organic acids such as acetic acid, propionic acid, hexanoic acid, cyclopentanepropionic acid, glycolic acid, pyruvic acid, lactic acid, malonic acid, succinic acid, malic acid, maleic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, 3-(4-hydroxybenzoyl)benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, 1,2-ethane-disulfonic acid, 2-hydroxyethanesulfonic acid, benzenesulfonic acid, 4-chlorobenzenesulfonic acid, 2-naphthalenesulfonic acid, 4-toluenesulfonic acid, camphorsulfonic acid, 4-methylbicyclo[2.2.2]-oct-2-ene-1-carboxylic acid, glucoheptonic acid, 4,4'-methylenebis-(3-hydroxy-2-ene-1-carboxylic acid), 3-phenylpropionic acid, trimethylacetic acid, tertiary butylacetic acid, lauryl sulfuric acid, gluconic acid, glutamic acid, hydroxynapthoic acid, salicylic acid, stearic acid, muconic acid, and the like; or

(2) salts formed when an acidic proton present in the parent compound either is replaced by a metal ion, e.g., an alkali metal ion, an alkaline earth ion, or an aluminum ion; or coordinates with an organic base such as ethanolamine, diethanolamine, triethanolamine, tromethamine, *N*-methylglucamine, and the like.

"Pro-drugs" means any compound which releases an active parent drug according to Formula (I) *in vivo* when such prodrug is administered to a mammalian subject. Prodrugs of a compound of Formula (I) are prepared by modifying functional groups present in the compound of Formula (I) in such a way that the modifications may be cleaved *in vivo* to release the parent compound. Prodrugs include compounds of Formula (I) wherein a hydroxy, amino, or sulfhydryl group in a compound of Formula (I) is bonded to any group that may be cleaved *in vivo* to regenerate the free hydroxyl, amino, or sulfhydryl group, respectively. Examples of prodrugs include, but are not limited to esters (e.g., acetate, formate, and benzoate derivatives), carbamates (e.g., N,N-dimethylaminocarbonyl) of hydroxy functional groups in compounds of Formula (I), and the like.

"Treating" or "treatment" of a disease includes:

- (1) preventing the disease, i.e. causing the clinical symptoms of the disease not to develop in a mammal that may be exposed to or predisposed to the disease but does not yet experience or display symptoms of the disease,
- (2) inhibiting the disease, i.e., arresting or reducing the development of the disease or its clinical symptoms, or
- (3) relieving the disease, i.e., causing regression of the disease or its clinical symptoms.

A "therapeutically effective amount" means the amount of a compound that, when administered to a mammal for treating a disease, is sufficient to effect such treatment for the disease. The "therapeutically effective amount" will vary depending on the compound, the disease and its severity and the age, weight, etc., of the mammal to be treated.

Compounds of this invention may be conveniently named with reference to their amino acid components in accordance with nomenclature conventional to the peptide field.

For example a dipeptide where n is 0, $R^1 = R^3 = R^4 = R^6 = H$, R^2 is 4-thiazolylmethyl, R^5 is (S,S)-1-methylpropyl and ZR^7 is benzyloxycarbonyl, is named as CBz-Ile-4-Taz-NHOH. With respect to the synthetic schemes A and B subsequently presented, 4-Taz (4-thiazolylalanine) represents AA_1 and Ile represents AA_2 .

A tripeptide where n is 1 and m is 0, $R^1 = R^3 = R^4 = R^6 = R^9 = H$, R^2 is 4-thiazolylmethyl, R^5 is (S,S)-1-methylpropyl, ZR^7 is 4-chlorobenzoyl and R^{10} is 4-fluorobenzyl is named 4-chlorobenzoyl-(4-fluorobenzyl)Gly-Ile-Taz-NHOH. With respect to the synthetic schemes A and B subsequently presented, 4-Taz represents AA_1 , Ile represents AA_2 and (4-fluorobenzyl)Gly represents AA_3 .

While the broadest definition of this invention is set forth in the first aspect of the present invention, certain compounds of formula (I) are preferred.

One class of compounds are the dipeptidic hydroxamic acids corresponding to compounds of Formula 1 where n is 0. Another class of compounds are the tripeptidic hydroxamic acids corresponding to compounds of Formula 1 where n is 1. For both the dipeptidic and tripeptidic hydroxamic acid compounds of the invention, particularly preferred are those where R^2 is heteroaralkyl, particularly 4-thiazolylmethyl and R^5 is (S,S)-1-methylpropyl. Preferably, R^2 , R^5 and R^9 are present in the naturally occurring amino acid configuration, i.e. derived from the (L) amino acids.

Another class of preferred compounds of Formula I is that where Z is $C(O)O$. Another class of preferred compounds of Formula I is that where Z is $S(O)_2$. Within each of these classes of compounds, more preferred are those where R^7 is aryl, aralkyl or heteroaryl, particularly halophenyl (e.g. 3,4-dibromophenyl, 2,5-dichlorophenyl or 2,4,5-trichlorophenyl), benzyl (optionally substituted) (e.g. benzyl or 3,4-dichlorobenzyl) or halothienyl (e.g. 4,5-dibromothien-2-yl). When Z is $C=O$,

also preferred are R^7 being aralkyl or heteroaralkyl, particularly benzyl or heteroarylmethyl substituted with halo, preferably one or two chloro.

Another class of preferred compounds is that where R^2 is aralkyl or heteroaralkyl. Within this class, more preferred are those compounds where R^2 is 3-indolyl methyl, 2-thienylmethyl, 4-imidazolylmethyl or 4-thiazolylmethyl, particularly 4-thiazolylmethyl.

Another class of preferred compounds are those where R^2 is (alkylene)-B-X where B is -O-, -NR⁸-, -S-, -C=O-, -CONR⁸-, -NR⁸CO₂-, -NR⁸SO₂- or -C(=NR⁸)NR⁸SO₂-(where R^8 is independently H or alkyl), and X is cycloalkyl, cycloalkylalkyl, aryl, aralkyl heteroaryl or heteroaralkyl. Particularly preferred are compounds where the alkylene group is methylene, B is -NR⁸CO₂ and X is aralkyl.

A fourth class of preferred compounds are those where R^5 is alkyl or phenyl, particularly propyl, 1-methylethyl, or (S,S)-1-methylpropyl. Within this class of compounds, particularly preferred are those where R^1 and R^4 are hydrogen and R^2 is heteroaralkyl, particularly 4-thiazolylmethyl.

Furthermore preferred compounds are those of group B, namely compounds of formula I as defined in the first aspect of the present invention wherein n is 0, or

(1) the compound of B wherein R^3 and R^6 are hydrogen.

(2) the compound of (1), wherein:
 R^2 is aralkyl or heteroaralkyl.

(3) the compound of (2) wherein:
Z is -C(O)O- or -S(O)₂-.

- (4) the compound of (3) wherein:
R² is optionally substituted benzyl or heteroaralkyl.
- (5) the compound of (4) wherein, R² is 4-t-butoxybenzyl, 3-chlorobenzyl, 3-indolyl methyl, 2-thienylmethyl, 4-imidazolylmethyl or 4-thiazolylmethyl.
- (6) the compound of (5) wherein R² is 4-thiazolylmethyl.
- (7) the compound of (6) wherein:
R⁷ is aryl, aralkyl, heteroaryl or heteroaralkyl.
- (8) the compound of (6) wherein:
Z is -C(O)O- and R⁷ is optionally substituted benzyl.
- (9) the compound of (7) wherein:
Z is -SO₂- and R⁷ is aryl or heteroaryl.
- (10) the compound of (8) or (9), wherein:
R¹ and R⁴ are hydrogen and R⁵ is alkyl.
- (11) the compound of (10) wherein R⁵ is (S,S)-1-methylpropyl.
- (12) The compound of (1), wherein:
R² is (alkylene)-B-X where B is -O-, -NR⁸-, -S(O)_n- (where n is 0, 1 or 2), -C=O, -CONR⁸-, -NR⁸CO₂-, -NR⁸SO₂- or -C(=NR⁸)NSO₂- (where R⁸ is H or alkyl), and X is cycloalkyl, cycloalkylalkyl, aryl, aralkyl, heteroaryl or heteroaralkyl.
- (13) The compound of (12), wherein:
Z is -C(O)O- or -S(O)₂-.

- (14) The compound of (13), wherein R^2 is $-\text{CH}_2\text{-B-X}$ and
B is $-\text{NHCO}_2\text{-}$ and X is benzyl.
- (15) The compound of (14) wherein:
5 R^7 is aryl or aralkyl.
- (16) The compound of (15), wherein:
 R^1 and R^4 are hydrogen and R^5 is alkyl.
- 10 (17) The compound of (16) wherein R^5 is (S,S)-1-methylpropyl.

Furthermore preferred compounds are these of group C, namely compounds of formula I as defined in the first aspect of the present invention wherein n is 1.

- 15 (1) The compound of C wherein m is 0 and R^3 and R^6 are hydrogen.
- (2) The compound of (1), wherein:
 R^2 is aralkyl or heteroaralkyl.
- 20 (3) The compound of (2), wherein:
Z is $-\text{C}(\text{O})\text{O-}$ or $-\text{S}(\text{O})_2\text{-}$.
- (4) The compound of (3), wherein:
 R^2 is optionally substituted benzyl or heteroaralkyl.
- 25 (5) The compound of (4) wherein R^2 is 4-t-butoxybenzyl, 3-chlorobenzyl, 3-indolyl methyl, 2-thienylmethyl, 4-imidazolylmethyl or 4-thiazolylmethyl.
- (6) The compound of (5) wherein R^2 is 4-thiazolylmethyl.
- 30

- (7) The compound of (6) wherein:
R⁷ is aryl, aralkyl, heteroaryl or heteroaralkyl.
- 5 (8) The compound of (7) wherein:
Z is -C(O)O- and R⁷ is benzyl.
- (9) The compound of (7) wherein:
Z is -SO₂- and R⁷ is aryl.
- 10 (10) The compound of (8) or (9), wherein:
R¹ and R⁴ are hydrogen and R⁵ is alkyl.
- (11) The compound of (10) wherein R⁵ is (S,S)-1-methylpropyl.
- 15 (12) The compound of (1), wherein:
R² is (alkylene)-B-X where B is -O-, -NR⁸-, -S-, -C=O, -CONR⁸-, -NR⁸CO₂-,
-NSO₂- or -C(=NR⁸)NSO₂-(where R⁸ is H or alkyl), and X is cycloalkyl,
cycloalkylalkyl, aryl, aralkyl heteroaryl or heteroaralkyl.
- 20 (13) The compound of (12), wherein:
Z is -C(O)O- or -S(O)₂-.
- (14) The compound of (13), wherein R² is CH₂-B-X and
B is -NHCO₂- and X is benzyl.
- 25 (15) The compound of (14) wherein:
R⁷ is aryl or aralkyl.
- (16) The compound of (15), wherein:
30 R¹ and R⁴ are hydrogen and R⁵ is alkyl.

(17) The compound of (16) wherein R⁵ is (S,S)-1-methylpropyl.

Representative compounds of this invention that have been prepared according to the Schemes and Examples are as follows:

I. Dipeptidic hydroxamic acids of Formula (I) where n = 0, R¹ = R³ = R⁴ = R⁶ = hydrogen and ZR⁷ is benzyloxycarbonyl and other groups are as defined below are:

TABLE I

Compound	Example	St*	R2	St**	R5	m/e
1	I	S	4-thiazolylmethyl	S	phenyl	455
2	I	S	4-thiazolylmethyl	S	2-carboxyethyl	451
3	I	S	4-thiazolylmethyl	S	isobutyl	435
4	I	S	4-thiazolylmethyl	S	n-butyl	435
5	I	S	4-thiazolylmethyl	S	ethyl	407
6	I	S	4-thiazolylmethyl	S	2-phenethyl	483
7	I	S	4-thiazolylmethyl	S	3-indolylmethyl	508
8	I	S	4-thiazolylmethyl	S	hydroxymethyl	409
9	I	S	4-thiazolylmethyl	S,R	1-benzyloxyethyl	513
10	I	S	4-thiazolylmethyl	S	4-fluorobenzyl	487
11	I	S	4-thiazolylmethyl	S	benzyl	469
12	I	S	4-thiazolylmethyl	S	methyl	393
13	I	S	4-thiazolylmethyl	S	1-naphthylmethyl	519
14	I	S	(E)-styrylmethyl	S,S	1-methylpropyl	454
15	I	S	2-bromobenzyl	S,S	1-methylpropyl	506
16	I	S	3-thienylmethyl	S,S	1-methylpropyl	434
17	I	S	5-(2-bromo-thienyl)methyl	S,S	1-methylpropyl	511
18	I	S	2-furylmethyl	S,S	1-methylpropyl	418
19	I	S	3-benzothienylmethyl	S,S	1-methylpropyl	484
20	I	S	4-fluorobenzyl	S,S	1-methylpropyl	446
21	I	S	benzyloxymethyl	S,S	1-methylpropyl	458
22	I	S,R	1-benzyloxyethyl	S,S	1-methylpropyl	472
23	I	S	3-chlorobenzyl	S,S	1-methylpropyl	462
24	I	S	4-thiazolylmethyl	S	cyclopropylmethyl	433
25	I	S	4-thiazolylmethyl	S	cyclohexyl	461
26	I	S	4-thiazolylmethyl	S	2-methylthioethyl	453
27	I	S	4-thiazolylmethyl	S	carbamoylmethyl	436
28	I	R	3-indolylmethyl	S	2-methylthioethyl	485
29	I	R	3-indolylmethyl	S	butyl	467
30	I	R	3-indolylmethyl	S	phenyl	487
31	I	S	3-indolylmethyl	S,S	1-methylpropyl	466

Compound	Example	St*	R2	St**	R5	m/e
32	I	S	3-indolylmethyl	S	1-naphthylmethyl	551
33	I	S	3-indolylmethyl	R	2-naphthylmethyl	551
34	I	S	3-indolylmethyl	R	2-naphthylmethyl	551
35	I	S	3-indolylmethyl	S	2-naphthylmethyl	551
36	I	S	3-indolylmethyl	S	2-phenethyl	515
37	I	S	3-indolylmethyl	S	4-fluorobenzyl	519
38	I	S	3-indolylmethyl	S	ethyl	439
39	I	S	3-indolylmethyl	S	isopropyl	453
40	I	S	3-indolylmethyl	R	2-carboxyethyl	483
41	I	R	3-indolylmethyl	R	2-(N-hydroxycarbamoyl)ethyl	498
42	I	S	(1-benzyl-1H-imidazol-4-yl)methyl	S,S	1-methylpropyl	508
43	I	S	4-(2,6-dichlorobenzyloxy)benzyl	S,S	1-methylpropyl	602
44	I	S	2-cyclohexyloxy-carbonylethyl	S,S	1-methylpropyl	492
45	I	S	2-phenethyl	S,S	1-methylpropyl	442
46	I	S	4-methylbenzylthiomethyl	S,S	1-methylpropyl	488
47	I	S	2-naphthylmethyl	S,S	1-methylpropyl	478
48	I	S	4-benzyloxycarbonylaminobutyl	S,S	1-methylpropyl	543
49	I	S	4-methoxybenzyl	S,S	1-methylpropyl	458
50	I	S	4-benzyloxybenzyl	S,S	1-methylpropyl	534
51	I	S	4-methoxybenzylthiomethyl	S,S	1-methylpropyl	504
52	I	S	2-(4-methylbenzyl)-thioethyl"	S,S	1-methylpropyl	502
53	I	R	4-fluorobenzyl	S,S	1-methylpropyl	446
54	I	R	(1-benzyl-1H-imidazol-4-yl)methyl	S,S	1-methylpropyl	509
55	I	S	4-(2-chlorobenzyl-carboxamido)butyl	S,S	1-methylpropyl	577
56	I	S	(3-benzyloxymethyl-3H-imidazol-4-yl)methyl	S,S	1-methylpropyl	539
57	I	S	(1H-imidazol-4-yl)methyl	S,S	1-methylpropyl	419
58	I	S	2-pyridylmethyl	S,S	1-methylpropyl	429
59	I	S	3-chlorobenzyl	S,S	1-methylpropyl	462
60	I	S	3,4-dichlorobenzyl	S,S	1-methylpropyl	496
61	I	S	4-nitrobenzyl	S,S	1-methylpropyl	473
62	I	S	4-bromobenzyl	S,S	1-methylpropyl	506

Compound	Example	St*	R2	St**	R5	m/e
63	I	S	3-trifluoromethylbenzyl	S,S	1-methylpropyl	496
64	I	S	4-phenylbenzyl	S,S	1-methylpropyl	504
65	I	S	indan-2-yl	S,S	1-methylpropyl	454
66	I	S	benzyloxycarbonylaminoethyl	S,S	1-methylpropyl	501
67	I	S	4-(4-tolylsulfonamido)butyl	S,S	1-methylpropyl	563
68	I	S	2-(4-nitrophenyl)ethyl	S,S	1-methylpropyl	499
69	I	S	3-(4-tosylguanidino)propyl	S,S	1-methylpropyl	591
70	I	S	4-tertbutylthiobenzyl	S,S	1-methylpropyl	516
71	I	S	3-tertbutoxybenzyl	S,S	1-methylpropyl	500
72	I	S	4-tertbutoxycarbonylmethylbenzyl	S,S	1-methylpropyl	558
73	I	S	4-carbamoylbenzyl	S,S	1-methylpropyl	471
74	I	S	4-acetamidobenzyl	S,S	1-methylpropyl	485
75	I	S	2-propargyl	S,S	1-methylpropyl	376
76	I	S	2-allyl	S,S	1-methylpropyl	378
77	I	S	4-thiazolylmethyl	S,S	1-methylpropyl	435

St* - Stereochemistry at carbon attached to R² and of R² substituent (if it has an independent chiral centre)

- 5 St** - Stereochemistry at carbon attached to R⁵ and of R⁵ substituent (if it has an independent chiral centre)

II. Dipeptidic hydroxamic acids of Formula (I) where $n = 0$, $R^1 = R^3 = R^4 = R^6 =$ hydrogen and R^2 is (S,S)-1-methylpropyl and other groups are as defined below are:

TABLE II

Compound	Example	St*	R2	ZR7	m/e
1	II	S	4-thiazolylmethyl	4-methoxybenzene-sulfonyl	471
2	II	S	4-thiazolylmethyl	benzenesulfonyl	441
3	II	S	4-thiazolylmethyl	2-chlorobenzenesulfonyl	474
4	II	S	4-thiazolylmethyl	3-chlorobenzenesulfonyl	474
5	II	S	4-thiazolylmethyl	4-chlorobenzenesulfonyl	474
6	II	S	4-thiazolylmethyl	2,4-dichlorobenzene-sulfonyl	508
7	II	S	4-thiazolylmethyl	4-fluorobenzenesulfonyl	458
8	II	S	4-thiazolylmethyl	3-nitrobenzenesulfonyl	485
9	II	S	4-thiazolylmethyl	4-methylbenzenesulfonyl	454
10	II	S	4-thiazolylmethyl	3-trifluoromethylbenzene-sulfonyl	508
11	II	S	4-thiazolylmethyl	4-bromo-2,5-difluorobenzenesulfonyl	556
12	II	S	4-thiazolylmethyl	5-dimethylamino-1-naphthalenesulfonyl	533
13	II	S	4-thiazolylmethyl	4-isopropylbenzene-sulfonyl	483
14	II	S	4-thiazolylmethyl	2,4,6-trimethylbenzene-sulfonyl	483
15	II	S	4-thiazolylmethyl	4-methoxy-2,3,6-trimethylbenzenesulfonyl	512
16	II	S	4-thiazolylmethyl	2,3,4,5,6-pentamethylbenzenesulfonyl	511
17	II	S	4-thiazolylmethyl	4,5-dibromothiophene-2-sulfonyl	604
18	II	S	4-thiazolylmethyl	3,4-dibromobenzene-sulfonyl	599
19	II	S	4-thiazolylmethyl	4-chloro-2,5-dimethylbenzenesulfonyl	503
20	II	S	4-thiazolylmethyl	2,4,5-trichlorobenzene-sulfonyl	542
21	II	S	4-thiazolylmethyl	4-bromo-2-(trifluoromethoxy)benzenesulfonyl	604
22	II	S	4-thiazolylmethyl	4-methoxynaphthalene-1-sulfonyl	521
23	II	S	4-thiazolylmethyl	4-benzenesulfonylthiophene-2-sulfonyl	586
24	II	S	4-thiazolylmethyl	2-chloro-4-(trifluoromethyl)benzenesulfonyl	543

Compound	Example	St*	R2	ZR7	m/e
25	II	S	4-thiazolylmethyl	5-chloronaphthalene-1-sulfonyl	525
26	II	S	4-thiazolylmethyl	2,2,5,7,8-pentamethyl-chroman-6-sulfonyl	567
27	II	S	4-thiazolylmethyl	4-methoxy-2-nitro-benzenesulfonyl	516
28	II	S	4-thiazolylmethyl	1-naphthalenesulfonyl	491
29	II	S	4-thiazolylmethyl	5-methoxybenzofuran-2-sulfonyl	511
30	II	S	4-thiazolylmethyl	4-tert-amylbenzene-sulfonyl	511
31	II	S	4-thiazolylmethyl	4-(4-chlorophenoxy)-benzenesulfonyl	566
32	II	S	4-thiazolylmethyl	2-(pyrid-2-yl)thiophene-5-sulfonyl	523
33	II	S	4-thiazolylmethyl	2-{3-[1-methyl-5-(trifluoromethyl)pyrazolyl]}thiophene-5-sulfonyl	594
34	II	S	4-thiazolylmethyl	3,5-dimethylisoxazole-4-sulfonyl	460
35	II	S	4-thiazolylmethyl	benzofurazan-4-sulfonyl	482
36	III	S	3-indolylmethyl	benzylcarbamoyl	466
37	IV	S	3-indolylmethyl	phenylcarbamoyl	452
38	III	S	3-indolylmethyl	benzoyl	437
39	IV	S	3-indolylmethyl	2-fluorophenylcarbamoyl	470
40	III	S	3-indolylmethyl	2-methoxyphenyl-carbamoyl	482
41	III	S	3-indolylmethyl	cyclohexylcarbamoyl	458
42	III	S	3-indolylmethyl	2-phenethylcarbamoyl	480
43	III	S	3-indolylmethyl	3,5-dichlorophenyl-carbamoyl	520
44	IV	S	3-indolylmethyl	2,4-dichlorobenzoyl	505
45	IV	S	3-indolylmethyl	4-trifluoromethylbenzoyl	505
46	IV	S	3-indolylmethyl	3-methylbenzoyl	451
47	IV	S	3-indolylmethyl	cyclohexanoyl	443
48	IV	S	4-thiazolylmethyl	3-furanoyl	395
49	IV	S	4-thiazolylmethyl	cyclopentanoyl	397
50	IV	S	4-thiazolylmethyl	4-aminobenzoyl	420
51	IV	S	4-thiazolylmethyl	4-hydroxybenzoyl	420
52	IV	S	4-thiazolylmethyl	4-dimethylaminobenzoyl	448
53	V	S	4-thiazolylmethyl	3,4-dichlorobenzoyloxy-carbonyl	503
54	V	S	4-thiazolylmethyl	3-chlorobenzoyloxy-carbonyl	468

Compound	Example	St*	R2	ZR7	m/e
55	V	S	4-thiazolylmethyl	3-nitrobenzyloxycarbonyl	480
56	II	S	4-thiazolylmethyl	2-(1-naphthylethane)-sulfonyl	519
57	IV	S	4-thiazolylmethyl	(R/S)-2-(4-chlorophenoxy)propionyl	483
58	II	S	4-thiazolylmethyl	2-phenylbenzimidazole-5-sulfonyl	557
59	IV	S	4-thiazolylmethyl	3-isoquinolinecarbonyl	456
60	IV	S	4-thiazolylmethyl	(P/S)- α -hydroxy-3-nitrophenylacetyl	481
61	X	S	4-thiazolylmethyl	1-carbonylmethyl-2,3,4,6,7,8,9,10-octahydro-1H-pyrimidino[1,2-a]azepin-5-ylum bromide	494
62	IV	S	4-thiazolylmethyl	4-fluorophenoxyacetyl	452
63	II	S	4-thiazolylmethyl	4-phenylazobenzene-sulfonyl	545
64	VII	S	4-thiazolylmethyl	2-(4-chlorophenyl)ethyl-carbamoyl	482
65	VII	S	4-thiazolylmethyl	3-phenylpropylcarbamoyl	462
66	X	S	4-thiazolylmethyl	1-(4-(2-nitrophenyl)-piperazino)methyl-carbonyl	548
67	IX	S	4-thiazolylmethyl	2-(3-chlorophenylsulfon-amido)ethylcarbonyl	546
68	IV	S	4-thiazolylmethyl	4-vinylbenzoyl	431
69	IV	S	4-thiazolylmethyl	2-ethylsulfanylnicotinoyl	466
70	IV	S	4-thiazolylmethyl	(R/S)-2-hydroxy-3-phenylpropionyl	449
71	IV	S	4-thiazolylmethyl	1-hydroxycyclopropyl-carbonyl	385
72	IV	S	4-thiazolylmethyl	(R/S)-tetrahydrofuran-3-carbonyl	399
73	IV	S	4-thiazolylmethyl	(R)-(-)-2-oxo-4-thiazolidinecarbonyl	430
74	IV	S	4-thiazolylmethyl	(S)-2-hydroxy-2-(1H-imidazol-4-yl)propionyl	439
75	IV	S	4-thiazolylmethyl	5-chloro-2-thiophene-carbonyl	444
76	IV	S	4-thiazolylmethyl	(R/S)-1-hydroxy-(4-methoxyphenyl)acetyl	465
77	IV	S	4-thiazolylmethyl	(2,6-dihydroxypyrimidin-4-yl)acetyl	453
78	III	S	4-thiazolylmethyl	cyclohexylcarbamoyl	426
79	I	S	benzyloxycarbonylaminomethyl	benzyloxycarbonyl	501

Compound	Example	St*	R2	ZR7	m/e
80	II	S	benzyloxycarbonylaminomethyl	2,5-dichlorobenzene-sulfonyl	575
81	II	S	benzyloxycarbonylaminomethyl	3,4-dibromobenzene-sulfonyl	664
82	II	S	benzyloxycarbonylaminomethyl	4,5-dibromothiophene-2-sulfonyl	670
83	II	S	benzyloxycarbonylaminomethyl	2,5-dimethyl-4-chlorobenzenesulfonyl	569
84	II	S	benzoylaminomethyl	benzyloxycarbonyl	471
85	II	S	4-thiazolylmethyl	2,5-dichlorobenzene-sulfonyl	508

St* Stereochemistry at carbon attached to R²

III. Dipeptidic hydroxamic acids of Formula (I) where $n = 0$, $R^1 = R^3 = R^6 =$

5 hydrogen and ZR⁷ is benzyloxycarbonyl and other groups are as defined below are:

TABLE III

Compound	Example	St*	R2	R4 & R5	
1	I	S	3-indolylmethyl	propylene	451
2	I	S	3-indolylmethyl	pentylene	479
3	I	S	3-indolylmethyl	dimethyl	439

St* - Stereochemistry at carbon attached to R²

IV. Dipeptidic hydroxamic acids of Formula (I) where $n = 0$, $R^1 = R^3 = R^4 =$ hydrogen, R^2 is (S) 3-indolylmethyl and ZR^7 is benzyloxycarbonyl and other groups are as defined below are:

TABLE IV

Compound	Example	St*	R5	R6	m/e
1	I		H	methyl	425
2	I	S	2-carboxyethyl	methyl	497
3	I	S	methyl	methyl	439
4	I	S	isopropyl	methyl	467
5	I	S	benzyl	methyl	515
6	VI		H	2-methylpropyl	467
7	VI		H	4-pyridylmethyl	502

5

St* - Stereochemistry at carbon attached to R^5

V. Tripeptidic hydroxamic acids of Formula I where $n = 1$, $m = 0$, $R^1 = R^3 = R^4 = R^6 = H$, R^2 is (S) 4-thiazolylmethyl, R^5 is (S,S)-1-methylpropyl and other groups are as defined below are:

10

TABLE V

Compound	Example	St*	R9	ZR7	R10	m/e
1	VIII	S	2-methylthioethyl	benzyloxy-carbonyl	H	566
2	VIII	S	2-benzyloxy-carbonyl ethyl	benzyloxy-carbonyl	H	654
3	VIII	S	3-indolylmethyl	benzyloxy-carbonyl	H	621
4	VIII	S	2-benzyloxy-carbonyl ethyl	benzyloxy-carbonyl	H	654
5	VIII	S	4-fluorobenzyl	benzyloxy-carbonyl	H	600
6	VIII	S	benzyloxymethyl	benzyloxy-carbonyl	H	612
7	VIII	S	2-methylpropyl	benzyloxy-carbonyl	H	548
8	VIII	S	4-hydroxybenzyl	benzyloxy-carbonyl	H	598
9	VIII	S	benzyl	benzyloxy-carbonyl	H	582
10	IX	S	benzyl	2-chlorobenzyl-oxycarbonyl	H	616

Compound	Example	St*	R9	ZR7	R10	m/e
11	VIII	S	1-naphthyl-methyl	benzyloxycarbonyl	H	632
12	VIII	S	phenyl	benzyloxy-carbonyl	H	568
13	VIII	S	tert-butoxy-carbonylmethyl	benzyloxy-carbonyl	H	606
14	VIII	S,S	1-methylpropyl	benzyloxy-carbonyl	H	548
15	VIII	S	benzyl	benzyloxy-carbonyl	methyl	596
16	X		H	4-chloro-benzoyl	4-fluoro-benzyl	604
17	X		H	3-chloro-benzoyl	4-fluoro-benzyl	604
18	X		H	3-methyl-benzoyl	4-fluoro-benzyl	584
19	X		H	phenylcarbamoyl	4-fluoro-benzyl	585
20	IX	S	benzyl	phenyl-carbamoyl	H	567
21	X		H	4-nitrobenzoyl	4-fluoro-benzyl	615
22	X		H	4-trifluoro-methylbenzoyl	4-fluoro-benzyl	638
23	X		H	2-methoxy-benzoyl	4-fluoro-benzyl	600
24	X		H	4-(N-hydroxy-carbamimidoyl) benzoyl	4-fluoro-benzyl	628
25	X		H	(R/S) 2-(4-chlorophenyl)-1-hydroxy-methylethyl	H	525
26	X		H	furfuryl	H	438
27	X		H	2-(4-morpholino)ethyl	H	471
28	X		H	2-pyridylmethyl	H	449
29	X		H	3-(1-imidazolyl)propyl	H	466
30	X		H	2,3-dimethoxy-benzyl	H	508
31	X		H	3-nitrobenzyl	H	493
32	X		H	2-(4-chlorophenethyl)	H	496
33	IX	S	4-fluorobenzyl	4-tertbutyl-benzenesulfonyl	H	662

Compound	Example	St*	R9	ZR7	R10	m/e
34	IX	S	4-fluorobenzyl	3-chlorobenzene-sulfonyl	H	640
35	IX	S	4-fluorobenzyl	2,4-dichloro-benzenesulfonyl	H	674
36	IX	S	4-fluorobenzyl	4-methoxy-benzenesulfonyl	H	636
37	IX	S	4-fluorobenzyl	4-methylbenzene-sulfonyl	H	620
38	IX	S	benzyl	3-chlorobenzene-sulfonyl	H	622
39	X		H	2,3-dichloro-benzenesulfonyl	4-fluorobenzyl	674
40	X		H	4-tertbutyl-benzenesulfonyl	4-fluorobenzyl	662

St* - Stereochemistry at carbon attached to R⁹ and of R⁹ substituent (if it has an independent chiral centre)

5

VI. Miscellaneous tripeptidic hydroxamic acid compounds of the invention where n = 1, m = 1, R¹ = R³ = R⁴ = R⁶ = H, R² is (S)-4-thiazolylmethyl and R⁵ is (S,S)-1-methylpropyl, and other groups are as defined below are include:

10

TABLE VI

Compound	Example	R9	ZR7	R10	m/e
1	IX	H	3-bromobenzenesulfonyl	H	591
2	IX	H	3-nitrobenzenesulfonyl	H	557

Other miscellaneous compounds with R² and R³ forming an alkylene or heteroalkylene chain include CBz-Ile-Pro-NHOH (m/e = 378) and CBz-Ile-Thz-NHOH (m/e = 396). Thz is thiazolidine-4-carboxylic acid.

15

Other miscellaneous compounds with R⁵ and R⁶ forming an alkylene chain include CBz-Pro-Trp-NHOH (m/e = 451).

20

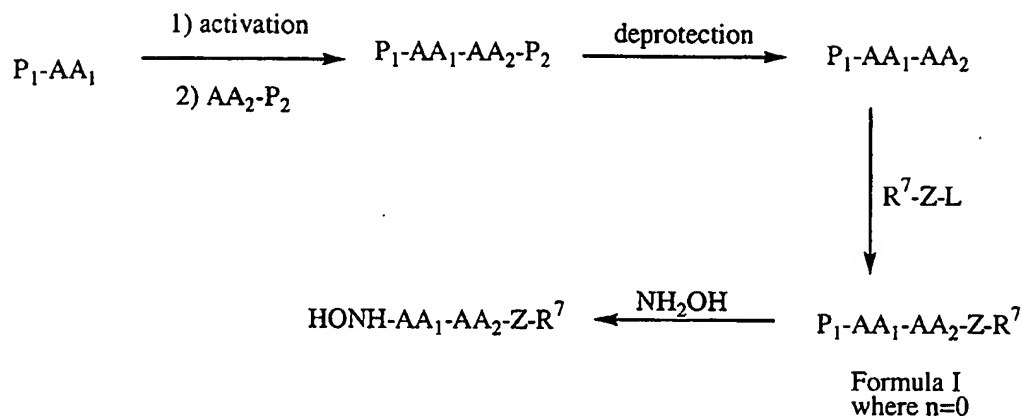
Compounds of this invention may be made by several synthetic methods as described below.

The starting materials and reagents used in preparing these compounds are either available from commercial suppliers such as Aldrich Chemical Co., (Milwaukee, Wisconsin, USA), Bachem (Torrance, California, USA), Emka-Chemie, or Sigma (St. Louis, Missouri, USA) or are prepared by methods known to those skilled in the art following procedures set forth in references such as *Fieser and Fieser's Reagents for Organic Synthesis*, Volumes 1-15 (John Wiley and Sons, 1991); *Rodd's Chemistry of Carbon Compounds*, Volumes 1-5 and Supplementals (Elsevier Science Publishers, 1989), *Organic Reactions*, Volumes 1-40 (John Wiley and Sons, 1991), *March's Advanced Organic Chemistry*, (John Wiley and Sons, 4th Edition), and *Larock's Comprehensive Organic Transformations* (VCH Publishers Inc., 1989). In particular, a variety of natural and unnatural amino acids in various protected forms are available from specialty chemical suppliers such as Novabiochem Inc. (La Jolla, CA), Advanced Chemtech Inc. (Louisville, KY), Synthetech Inc. (Albany, OR), Bachem Inc. (Torrance, CA). These schemes are merely illustrative of some methods by which the compounds of this invention can be synthesized, and various modifications to these schemes can be made and will be suggested to one skilled in the art having referred to this disclosure.

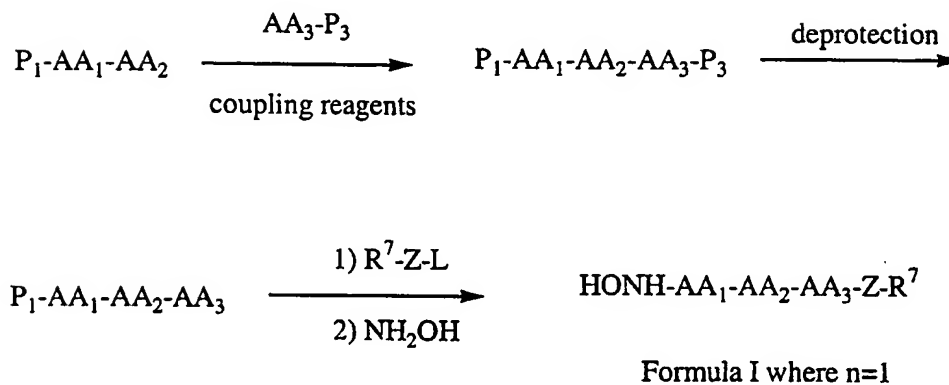
The starting materials and the intermediates of the reaction may be isolated and purified if desired using conventional techniques, including but not limited to filtration, distillation, crystallization, chromatography, and the like.

The compounds of the invention are C-terminal hydroxamic derivatives of natural and unnatural di- and tripeptides further functionalized at the N-terminus. They may be made by initially assembling the peptide precursor followed by deprotection (as necessary) and functionalization of the C- and N-termini. The peptide precursors are made by methods known to those of skill in the art of peptide synthesis, including solution phase chemistries and solid phase synthesis, see Solid Phase Peptide Synthesis: A Practical Approach by E. Atherton and R.C. Sheppard (Oxford University Press, 1989)

In general, as shown in Schemes A and B, a C-terminal protected amino acid P_1-AA_1 is coupled to an N-protected amino acid AA_2-P_2 to give a compound $P_1-AA_1-AA_2-P_2$. Coupling is accomplished by activating the C-terminus of AA_2-P_2 and
5 condensation with the amine of P_1-AA_1 . Activating reagents and coupling conditions are well known to one of skill in the art and include carbodiimide mediated coupling or formation of N-hydroxysuccinimide esters followed by acylation. For synthesis of the dipeptidic hydroxamic acids, protecting group removal and N-terminal
10 functionalization with R^7-Z-L (in either order) is effected to give a dipeptide precursor that is converted to a hydroxamic compound of the invention, typically by treatment with hydroxylamine. For synthesis of the tripeptidic hydroxamic acids, compound $P_1-AA_1-AA_2-P_2$ is selectively N-deprotected to give $P_1-AA_1-AA_2$ which is then coupled to an N-protected amino acid AA_3-P_3 . Deprotection, N-terminal functionalization and
15 treatment with hydroxylamine as shown in Scheme B then gives tripeptide hydroxamic acids of Formula I.

Scheme ASynthesis of dipeptidic hydroxamic acids

5

Scheme BSynthesis of tripeptidic hydroxamic acids

10

With reference to the nomenclature in Formula I, AA₁ corresponds to -C(=O)-CR¹R²-NR³-, AA₂ corresponds to -C(=O)-CR⁴R⁵-NR⁶ and AA₃ corresponds to -C(=O)-CH(R⁹)-(CH₂)_m-NR¹⁰. P₁, P₂ and P₃ represent protecting groups.

15

In particular, compounds of this invention may be prepared by solid phase synthesis. Initially, an N-protected amino acid P₁-AA₁ is attached to a solid phase resin

via its C-terminus. Typical solid phase resins include chloromethylated and hydroxymethylated resins such as the 4-hydroxymethyl-phenylacetamidomethyl resin (Pam resin) and the 4-benzyloxybenzyl alcohol resin (Wang resin) available from Advanced Chemtech, Louisville, Kentucky, U.S.A. and the pegylated polystyrene resin, ArgoGel-OH™, resin from Argonaut Technologies Inc. (Belmont, CA). Preferred chloromethyl resins include the styrene/divinylbenzene resins known as Merrifield resins available from Aldrich Chemical Company, Milwaukee, Wisconsin, U.S.A.

Amino acid building blocks, AA₂ and AA₃ are then attached sequentially using iterative coupling and deprotection steps well known to one of skill in the art. Coupling reactions are done under conventional peptide coupling conditions, typically in an anhydrous inert aprotic polar solvent (e.g. dimethyl formamide, acetonitrile, tetrahydrofuran, dichloromethane etc.) using auxiliary coupling reagents, e.g., carbodiimides such as 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDAC), diisopropylcarbodiimide (DIC), diimidazoles such as carbonyldiimidazole, triazoles such as hydroxybenzotriazole (HOBt) or other carboxyl activating groups such as N-hydroxysuccinimide, in the presence of an tertiary organic base such as 4-dimethylaminopyridine, N-methylmorpholine or triethylamine.

The protecting groups employed depend on the group being protected and are also known to those of skill in the art. Representative protecting groups may be found in Protective Groups in Organic Chemistry, J.F.W. McOmie (London, Plenum Press, (1973)) and Protective Groups in Organic Chemistry, T.W. Greene and P.G. Wuts (John Wiley and Sons, (1991)). A favoured N-protecting group is the fluorenylmethoxycarbonyl (Fmoc) group.

After assembly of the entire peptide skeleton, the protecting group at the N-terminus is removed. In certain cases the N-terminal protecting group may correspond to ZR⁷, obviating the need for protecting group removal. Functionalization at the N-terminus is effected by subsequent treatment with a compound R⁷-Z-L, where L is a

leaving group under nucleophilic displacement conditions. The conditions used for protecting group removal vary depending on the protecting group. Acid sensitive protecting groups such as tert-butoxycarbonyl (t-BOC) are removed with mild acid (e.g. trifluoroacetic acid) whereas base sensitive protecting groups such as 9-fluorenylmethoxycarbonyl (FMOC) are removed with mild organic base. Typical leaving groups L include halo, tosylate and mesylate.

Finally cleavage off the solid phase is accomplished by treatment with hydroxylamine to give compounds of Formula I.

As stated above, both natural and unnatural amino acids may be used in preparing the compounds of this invention. The natural amino acids and their abbreviations are well known and will not be repeated here. Some examples of unnatural amino acids and their abbreviations include, homoserine (hSer), homoserine lactone (hSerlac), homocysteine (Hcy), homoarginine (hArg), homocitrulline (Hci), penicillamine (Pen), N α -methylarginine (N-MeArg), norleucine (Nle), norvaline (Nval), norisoleucine (Nile), N-methylisoleucine (N-Melle), phenylglycine (PhG), t-butylglycine (Tle), hydroxyproline (Hyp), 3,4-dehydroproline (Δ -Pro), pyroglutamine (Pyr, Glp), ornithine (Orn), 2,3-diaminopropionic acid (2,3-DAP), 1-aminoisobutyric acid (1-Aib), 2-aminoisobutyric acid (2-Aib), 2-aminobutyric acid (2-Abu), 4-aminobutyric acid (4-Abu), 2,4-diaminobutyric acid (A2bu), α -aminosuberic acid (Asu), albizzin (Abz), β -cyclohexylalanine (Cha), 3-(1-naphthyl)alanine (1-Nal), 3-(2-naphthyl)alanine (2-Nal), citrulline (Cit), pipecolinic acid (Pip), 4-chlorophenylalanine (4-ClPhe), 4-fluorophenylalanine (4-FPhe), sarcosine (Sar), 4-thiazolylalanine (4-Taz), homophenylalanine (Hpa or hPhe), 2-thienylalanine (2-Thi), 3-benzothienylalanine (3-Bal), and 1-aminopropanecarboxylic acid (1-NCPC). A variety of unnatural amino acids are available from commercial vendors.

Compounds of this invention are useful to treat diseases associated with the excessive deposition of interstitial collagens, such as fibroproliferative diseases exemplified by interstitial pulmonary fibrosis, pericentral fibrosis, Symmers' fibrosis, perimuscular fibrosis, kidney fibrosis, endocardial sclerosis, hepatitis, acute
5 respiratory distress syndrome, arthritis, cystic fibrosis, surgical adhesions, tendon surgery, corneal scarring, scleroderma, chronic allograft rejection, hemodialysis shunt fibrosis and restenosis.

Compounds of this invention are inhibitors of procollagen C-proteinase and
10 thereby inhibit the C-terminal processing of types I, II and III collagens necessary for their ability to form insoluble collagen fibrils. Furthermore, selected compounds of the invention selectively inhibit procollagen C-proteinase over other collagen degrading enzymes such as collagenase-1, collagenase-2 and collagenase-3. As a result, compounds of this invention leave largely unaffected the natural resorption of
15 collagen mediated by collagenase-1, collagenase-2 and collagenase-3. Due to this selectivity, such compounds are of greater therapeutic efficacy than nonselective inhibitors. In particular, preferred compounds of this invention inhibit procollagen C-proteinase with greater than 100 fold selectivity over collagenase-1 and collagenase-2 and the most selective compounds are more than a thousand fold more
20 selective. Selective inhibition of procollagen C-proteinase over collagenase-1 and collagenase-2 was demonstrated by the assays described in the Examples. Thereby, this invention allows the treatment of fibrotic diseases by administering to a patient an agent that selectively inhibits procollagen C-proteinase over collagenase-1, collagenase-2 and collagenase-3. The inhibition may be 10 fold more selective,
25 preferably 100 fold more selective and most preferably 1000 fold more selective.

The ability of the compounds of Formula (I) to inhibit procollagen C-proteinase activity, may be demonstrated by a variety of *in vitro* assays known to those of ordinary skill in the art, such as the assay described in more detail in

Example XIII. The selectivity against collagenase enzymes may be determined by testing as described in Example XIV.

5 The *in vivo* efficacy of compounds of Formula (I) against fibrotic disease and the overproduction and deposition of collagen may be shown by numerous animal models including the mouse bleomycin induced pulmonary fibrosis model (S.H. Phan et.al. "Bleomycin-induced Pulmonary Fibrosis," Am. Rev. Respir. Dis., 124:428-434 (1981) and P.F. Piguet et al. "Effective Treatment of the Pulmonary Fibrosis Elicited in Mice by Bleomycin or Silica with anti-CD-11 Antibodies," Am. Rev. Resp. Dis., 10 147:435-441 (1993)) the sponge implant model (E.N. Unemori et al. "Human Relaxin Decreases Collagen Accumulation In Vivo in Two Rodent Models of Fibrosis," J. Invest. Dermatol., 101:280-285 (1993), the carbon tetrachloride or NDMU induced renal fibrosis model, as well as other animal models cited in WO 97/05865 ("C-Proteinase Inhibitors for the Treatment of Disorders Relating to the 15 Overproduction of Collagen").

In general, the compounds of this invention will be administered in a therapeutically effective amount by any of the accepted modes of administration for agents that serve similar utilities. The actual amount of the compound of this 20 invention, i.e., the active ingredient, will depend upon numerous factors such as the severity of the disease to be treated, the age and relative health of the subject, the potency of the compound used, the route and form of administration, and other factors. The drug can be administered more than once a day, preferably once or twice a day.

25

Therapeutically effective amounts of compounds of formula I may range from approximately 0.05-35 mg per kilogram body weight of the recipient per day; preferably about 0.3-20 mg/kg/day. Thus, for administration to a 70 kg person, the dosage range would most preferably be about 21 mg to 1.4 g per day.

In general, compounds of this invention will be administered as pharmaceutical compositions by any one of the following routes: oral, systemic (e.g., transdermal, intranasal, pulmonary or by suppository), or parenteral (e.g., intramuscular, intravenous or subcutaneous) administration. The preferred manner of administration is systemic using a convenient daily dosage regimen which can be adjusted according to the degree of affliction.

Intranasal delivery is typically accomplished with dry powder formulations, liquid solutions or suspensions suitable for nebulization or with aerosol propellants suitable for use in a metered dose inhaler. Alternatively, drug substance may be associated with microspheres made of materials such as gelatin, dextran, collagen or albumin. The microspheres are conveniently delivered in freeze dried form with a nasal insufflator device or a pressurized aerosol cannister. Penetration enhancers such as amphiphilic steroids may also be used as additives to increase the systemic absorption of the drug into the tissue.

Effective administration may also be accomplished by pulmonary or respiratory delivery since polypeptides are readily absorbed through the cellular lining of the alveolar region of the mammalian lung. Advantageously, such administration frequently does not require the use of penetration enhancers as additives. Devices and methods for pulmonary delivery deep into the lung are described in U.S. Patent No. 5,780,014 and U.S. Patent No. 5,814,607.

Lastly, compounds may be systemically administered by transdermal delivery, which typically involves placing the drug on the surface of the skin and allowing it to permeate through the skin. Transdermal delivery devices employ a structure such as an adhesive patch or the like that serves as a reservoir for the drug and brings the drug into diffusive contact with the skin. In one general typ, the structure is a three dimensionally stable matrix known as a monolithic matrix. Such matrices are described in more detail in U.S. Patent Nos. 5,804,214, 5,149,538 and 4,956,171

which describe matrices made of polymers and copolymers of acrylic latexes, acrylic esters, methacrylic esters and vinyl acetates.

The choice of formulation depends on various factors such as the mode of
5 drug administration (e.g., for oral administration, formulations in the form of tablets, pills or capsules are preferred) and the bioavailability of the drug substance. Recently, pharmaceutical formulations have been developed especially for drugs that show poor bioavailability based upon the principle that bioavailability can be increased by increasing the surface area i.e., decreasing particle size. For example,
10 U.S. Pat. No. 4,107,288 describes a pharmaceutical formulation having particles in the size range from 10 to 1,000 nm in which the active material is supported on a crosslinked matrix of macromolecules. U.S. Pat. No. 5,145,684 describes the production of a pharmaceutical formulation in which the drug substance is pulverized to nanoparticles (average particle size of 400 nm) in the presence of a surface
15 modifier and then dispersed in a liquid medium to give a pharmaceutical formulation that exhibits remarkably high bioavailability.

The compositions are comprised of in general, a compound of formula (I) in combination with at least one pharmaceutically acceptable excipient. Acceptable
20 excipients are non-toxic, aide administration, and do not adversely affect the therapeutic benefit of the compound of formula (I). Such excipient may be any solid, liquid, semi-solid or, in the case of an aerosol composition, gaseous excipient that is generally available to one of skill in the art .

25 Solid pharmaceutical excipients include starch, cellulose, talc, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, magnesium stearate, sodium stearate, glycerol monostearate, sodium chloride, dried skim milk and the like. Liquid and semisolid excipients may be selected from glycerol, propylene glycol, water, ethanol and various oils, including those of petroleum, animal,
30 vegetable or synthetic origin, e.g., peanut oil, soybean oil, mineral oil, sesame oil, etc.

Preferred liquid carriers, particularly for injectable solutions, include water, saline, aqueous dextrose, and glycols.

Compressed gases may be used to disperse a compound of this invention in aerosol form. Inert gases suitable for this purpose are nitrogen, carbon dioxide, etc.

Other suitable pharmaceutical excipients and their formulations are described in *Remington's Pharmaceutical Sciences*, edited by E. W. Martin (Mack Publishing Company, 18th ed., 1990).

The amount of the compound in a formulation can vary within the full range employed by those skilled in the art. Typically, the formulation will contain, on a weight percent (wt%) basis, from about 0.01-99.99 wt% of a compound of formula I based on the total formulation, with the balance being one or more suitable pharmaceutical excipients. Preferably, the compound is present at a level of about 1-80 wt%. Representative pharmaceutical formulations containing a compound of formula I are described in Example 30.

EXAMPLES

Ac₂O capping solution - 19 mL Ac₂O, 9 mL DIPEA, 0.8 eq. HOBt, 400 mL NMP

Abbreviations
Ac₂O - acetic anhydride
DIPEA - diisopropylethylamine
HOBt - hydroxybenzotriazole
NMP - N-Methylpyrrolidinone
Fmoc - Fluorenylmethoxycarbonyl
BOC - t-butoxycarbonyl
DIC - diisopropylcarbodiimide
DMAP - 4-dimethylaminopyridine
HATU - O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate]
HOAT - 1-hydroxy-7-azabenzotriazole
TFA - trifluoroacetic acid

DMF - dimethylformamide

TIS - triisopropylsilane

CBz - benzyloxycarbonyl

THF - tetrahydrofuran

5 Taz - 4-thiazolylalanine

Ile - isoleucine

Su - succinimidyl

TEA - triethylamine

Trp - tryptophan

10 DMSO - dimethylsulfoxide

TMS - trimethylsilyl

DMEM-HG - Dulbeccos Modified Eagle Medium, high glucose

G418 - Genitacin

15 SYNTHESIS OF COMPOUNDS OF FORMULA I

Compounds of Formula (I) were made by solid phase synthesis using conventional methods as generally described in the following Examples. If necessary, the compounds prepared as described in Examples I-X were purified by reverse phase high pressure liquid chromatography on silica gel bonded
20 diisopropylphenethylsilane columns (Zorbax SB-Phenyl) using gradient elution with a mixed acetonitrile-water (1% TFA) solvent system at flow rates of approximately 1.5-2.0 mL/minute. They were directly tested in the PCP and collagenase assays described in Examples XIII and XIV without further purification. The compounds may be characterized using conventional means, including physical constants and spectral data.
25 In particular, they were analyzed by mass spectrometry using electron spray ionization.

Example I

General Experimental for CBz-AA₂-AA₁-NHOH

(Dipeptide Compounds of Formula I where ZR⁷ is benzyloxycarbonyl)

30

1. BOC-AA₁ or FMOC-AA₁

To ArgoGel-OH™ (Argonaut Technologies, Belmont, CA) in a empty solid phase extraction vial, fitted with a stopcock was added 3 eq. of either BOC or FMOC protected AA₁, 3 eq. of diisopropylcarbodiimide and 0.05 eq. of a 0.116 M solution

of dimethylaminopyridine in THF. Sufficient CH_2Cl_2 was added to swell the resin (~12.5 mL/gr of resin). [Alternatively, to the resin was added 3 eq. of either BOC or Fmoc protected AA_1 , 3 eq. of HATU, 3 eq. HOAt and 6 eq. of DIPEA. Sufficient NMP was added to swell the resin (~12.5 mL/gr of resin).] The reaction was then placed on a spinner and rotated overnight. The reaction was then filtered by suction filtration and washed three times with CH_2Cl_2 , three times with MeOH, once with 1:1 HOAc/ CH_2Cl_2 , three times with MeOH and then lastly three times with CH_2Cl_2 and dried to give BOC- AA_1 -resin or Fmoc- AA_1 resin. The unreacted resin sites were then capped off with an Ac_2O capping solution (19 mL Ac_2O , 9 mL DIPEA, 0.8 gr HOBt, 400 mL NMP) in a sufficient quantity added to swell the resin (~12.5 mL/gr of resin). The resin was then filtered and washed as above.

2. $\text{H}_2\text{N-AA}_1$ -resin

2a. For BOC protected material - The resulting resin from above (BOC- AA_1 -resin) was treated with a solution of 95/2.5/2.5 TFA/ H_2O /triisopropyl silane (TIS) for two hours. The reaction was then filtered by suction filtration and washed three times with CH_2Cl_2 , three times with MeOH, and then three times with CH_2Cl_2 to give $\text{H}_2\text{N-AA}_1$ -resin.

2b. For Fmoc protected material - The resulting resin from above (Fmoc- AA_1 -resin) was first washed with DMF then treated with a solution of 20% piperidine in DMF for 20 min. The reaction was then filtered by suction filtration and washed three times with CH_2Cl_2 , three times with MeOH, once with 1:1 HOAc/ CH_2Cl_2 , three times with MeOH and then lastly three times with CH_2Cl_2 , to give $\text{H}_2\text{N-AA}_1$ -resin.

3. CBz- AA_2 - AA_1 resin

To $\text{H}_2\text{N-AA}_1$ -resin was added 3 eq. of CBZ protected AA_2 , 3 eq. of diisopropylcarbodiimide and 0.05 eq. of a 0.116 M solution of

dimethylaminopyridine in THF. Sufficient CH_2Cl_2 was added to swell the resin (~12.5 mL/gr of resin). [Alternatively, to the resin was added 3 eq. of CBz protected AA_2 , 3 eq. of HATU, 3 eq. HOAt and 6 eq. of DIPEA. Sufficient NMP is added to swell the resin (~12.5 mL/gr of resin).] The reaction was then placed on a spinner and rotated overnight. The reaction was then filtered by suction filtration and washed three times with CH_2Cl_2 , three times with MeOH, once with 1:1 HOAc/ CH_2Cl_2 , three times with MeOH and then lastly three times with CH_2Cl_2 to give CBz- AA_2 - AA_1 -resin.

10 4. Removal of protecting groups

If AA_1 or AA_2 contains an acid labile side chain protecting group that is to be removed, the resulting resin (CBz- AA_2 - AA_1 -resin) was treated with a solution of 95/2.5/2.5 TFA/ H_2O /triisopropyl silane (TIS) for 2 hours. The reaction was then filtered by suction filtration and washed three times with CH_2Cl_2 , three times with MeOH, and then three times with CH_2Cl_2 .

20 5. CBz- AA_2 - AA_1 -NHOH

CBz- AA_2 - AA_1 -resin was first washed with THF. Sufficient THF was added to swell the resin (~12.5 mL/gr of resin) then 25 eq. of 50% aq. NH_2OH was added and the reaction was rotated for two days. The reaction was then filtered by suction filtration and washed with CH_2Cl_2 , MeOH, and then CH_2Cl_2 . The filtrate was concentrated under vacuum to obtain CBz- AA_2 - AA_1 -NHOH.

Example IIGeneral Experimental for R^7 -SO₂-AA₂-AA₁-NHOH

(Dipeptide compounds of Formula I where Z is -SO₂-)

5 1. FMOC-AA₂-AA₁-resin

To H₂N-AA₁-resin was added 3 eq. of FMOC protected AA₂, 3 eq. of diisopropylcarbodiimide and 0.05 eq. of a 0.116 M solution of 4-dimethylaminopyridine in THF. Sufficient CH₂Cl₂ was added to swell the resin (~12.5 mL/gr of resin). The reaction was then placed on a spinner and rotated
10 overnight. The reaction was then filtered by suction filtration and washed three times with CH₂Cl₂, three times with MeOH, once with 1:1 HOAc/CH₂Cl₂, three times with MeOH and then lastly three times with CH₂Cl₂, to give FMOC-AA₂-AA₁-resin.

15 2. H₂N-AA₂-AA₁-resin

FMOC-AA₂-AA₁-resin was first washed with DMF then treated with a solution of 20% piperidine in DMF for 20 min. The reaction was then filtered by suction filtration and washed three times with CH₂Cl₂, three times with MeOH, once with 1:1 HOAc/CH₂Cl₂, 3 times with MeOH and then lastly 3 times with
20 CH₂Cl₂, to give H₂N-AA₂-AA₁-resin.

3. R^7 -SO₂-AA₂-AA₁-resin

H₂N-AA₂-AA₁-resin was first washed with aq. dioxane, then 10 eq. of the desired sulfonyl chloride, R^7 -SO₂Cl, was added. Sufficient 90% aq. dioxane was
25 added to swell the resin (~12.5 mL/gr of resin). Then 20 eq. of diisopropylethylamine was added. The reaction was then placed on a spinner and rotated overnight. The reaction was then filtered by suction filtration and washed three times with CH₂Cl₂, three times with MeOH, once with 1:1 HOAc/CH₂Cl₂,

three times with MeOH and then lastly three times with CH₂Cl₂ to give R⁷-SO₂-AA₂-AA₁-resin.

4. R⁷-SO₂-AA₂-AA₁-NHOH

R⁷-SO₂-AA₂-AA₁-resin was first washed with THF. Sufficient THF was added to swell the resin (~12.5 mL/gr of resin) then 25 eq. of 50% aq. NH₂OH was added and the reaction was rotated for two days. The reaction was then filtered by suction filtration and washed with CH₂Cl₂, MeOH, and then CH₂Cl₂. The filtrate was concentrated under vacuum to obtain R⁷-SO₂-AA₂-AA₁-NHOH.

10

Example III

General Experimental for R⁷NHCO-AA₂-AA₁-NHOH

(Dipeptide compounds of Formula I where Z is -CONH-)

15 1. R⁷-NHCO-AA₂-AA₁-resin

H₂N-AA₂-AA₁-resin was first washed with THF, then 3 eq. of the desired isocyanate, R⁷N=C=O, was added. Sufficient THF was added to swell the resin (~12.5 mL/gr of resin). The reaction was then placed on a spinner and rotated overnight. The reaction was then filtered by suction filtration and washed three times with CH₂Cl₂, three times with MeOH, once with 1:1 HOAc/CH₂Cl₂, three times with MeOH and then lastly three times with CH₂Cl₂ to give R⁷-NHCO-AA₂-AA₁-resin.

20

2. Removal of protecting groups

25

If AA₁ or AA₂ contains an acid labile side chain protecting group that is to be removed, the resulting resin (R⁷-NHCO-AA₂-AA₁-resin) was treated with a solution of 95/2.5/2.5 TFA/H₂O/triisopropyl silane (TIS) for 2 hours. The reaction

was then filtered by suction filtration and washed three times with CH_2Cl_2 , three times with MeOH, and then three times with CH_2Cl_2 .

3. $\text{R}^7\text{-NHCO-AA}_2\text{-AA}_1\text{-NHOH}$

5 $\text{R}^7\text{NHCO-AA}_2\text{-AA}_1\text{-resin}$ was first washed with THF. Sufficient THF was added to swell the resin (~12.5 mL/gr of resin) then 25 eq. of 50% aq. NH_2OH was added and the reaction was rotated for two days. The reaction was then filtered by suction filtration and washed with CH_2Cl_2 , MeOH, and then CH_2Cl_2 . The filtrate was concentrated under vacuum to obtain $\text{R}^7\text{NHCO-AA}_2\text{-AA}_1\text{-NHOH}$.

10

The dipeptide hydroxamic acids in Table II and Table V where Z is -CONH- were prepared using the procedure of Example 3.

Example IV

15

General Experimental for $\text{R}^7\text{-CO-AA}_2\text{-AA}_1\text{-NHOH}$

(Dipeptide compounds of Formula I where Z is -CO-)

1. $\text{R}^7\text{-CO-AA}_2\text{-AA}_1\text{-resin}$

20 To $\text{H}_2\text{N-AA}_2\text{-AA}_1\text{-resin}$ was added sufficient CH_2Cl_2 to swell the resin (~12.5 mL/gr of resin). 3 eq. of the desired acid chloride, R^7COCl , and 3 eq. of Et_3N were then added. A second alternative was add to the CH_2Cl_2 swollen resin 3 eq. of the desired carboxylic acid, $\text{R}^7\text{CO}_2\text{H}$, 3 eq. of diisopropylcarbodiimide and 0.05 eq. of a 0.116 M solution of dimethylaminopyridine in THF. A third alternative was to swell the resin with sufficient CH_3CN (~12.5 mL/gr of resin) and couple the
25 desired acid chloride, R^7COCl (3 eq.) in the presence of 6 eq. of trimethylsilylcyanide. In all cases the reaction was then placed on a spinner and rotated overnight. The reaction was then filtered by suction filtration and washed three times with CH_2Cl_2 , three times with MeOH, once with 1:1 HOAc/ CH_2Cl_2 ,

three times with MeOH and then lastly three times with CH₂Cl₂ to give R⁷-CO-AA₂-AA₁-resin.

2. Removal of protecting groups

5 If AA₁ or AA₂ contains an acid labile side chain protecting group that is to be removed, the resulting resin (R⁷-CO-AA₂-AA₁-resin) was treated with a solution of 95/2.5/2.5 TFA/H₂O/triisopropyl silane (TIS) for 2 hours. The reaction was then filtered by suction filtration and washed three times with CH₂Cl₂, three times with MeOH, and then three times with CH₂Cl₂.

10

3. R⁷-CO-AA₂-AA₁-NHOH

R⁷-CO-AA₂-AA₁-resin was first washed with THF. Sufficient THF was added to swell the resin (~12.5 mL/gr of resin) then 25 eq. of 50% aq. NH₂OH was added and the reaction was rotated for two days. The reaction was then filtered by
15 suction filtration and washed with CH₂Cl₂, MeOH, and then CH₂Cl₂. The filtrate is concentrated under vacuum to obtain R⁷-CO-AA₂-AA₁-NHOH.

Example V

General experimental for R⁷-OC(=O)-AA₂-AA₁-NHOH

20

(Dipeptide compounds of Formula I where Z is -C(O)O-)

1. R⁷-OCO-AA₂-AA₁-resin.

To H₂N-AA₂-AA₁-resin was added 3 eq. of the desired succinimidylcarbonate, R⁷OC(=O)NHS, 3 eq. Et₃N and 0.05 eq. of a 0.116 M
25 solution of 4-dimethylaminopyridine in THF. Sufficient CH₂Cl₂ was added to swell the resin (~12.5 mL/gr of resin). Another alternative was to add to the resin 10 eq. of the desired chloroformate, R⁷OCOCl, and 20 eq. diisopropylethylamine followed by

sufficient 90% aq. dioxane to swell the resin (~12.5 mL/gr of resin)]. In either case, the reaction was then placed on a spinner and rotated overnight. The reaction was then filtered by suction filtration and washed three times with CH₂Cl₂, three times with MeOH, once with 1:1 HOAc/CH₂Cl₂, three times with MeOH and lastly three
5 times with CH₂Cl₂ to give R⁷-OCO-AA₂-AA₁-resin.

2. Removal of protecting groups.

If AA₁ or AA₂ contains an acid labile side chain protecting group that is to be removed, the resulting resin (R⁷-OCO-AA₂-AA₁-resin) was treated with a
10 solution of 95/2.5/2.5 TFA/H₂O/triisopropyl silane (TIS) for 2 hours. The reaction was then filtered by suction filtration and washed three times with CH₂Cl₂, three times with MeOH, and then three times with CH₂Cl₂.

3. R⁷-OCO-AA₂-AA₁-NHOH

15 R⁷-OCO-AA₂-AA₁-resin was first washed with THF. Sufficient THF was added to swell the resin (~12.5 mL/gr of resin) and 25 eq. of 50% aq. NH₂OH is added and the reaction was rotated for two days. The reaction was then filtered by suction filtration and washed with CH₂Cl₂, MeOH, and then CH₂Cl₂. The filtrate was concentrated under vacuum to obtain R⁷-OCO-AA₂-AA₁-NHOH.

20

Example VI

General experimental for CBz-NR⁶-CH₂CO-AA₁-NHOH

1. BrCH₂CO-AA₁-resin

25 To H₂N-AA₁-resin was added 12 eq. of bromoacetic acid and 13 eq. of diisopropylcarbodiimide. Sufficient CH₂Cl₂ was added to swell the resin (~12.5 mL/gr of resin). The reaction was then placed on a spinner and rotated for 2 hrs.

The reaction was filtered by suction filtration and washed three times with CH_2Cl_2 , three times with DMSO and lastly three times with CH_2Cl_2 to give $\text{BrCH}_2\text{CO-AA}_1$ -resin.

5 2. $\text{R}^6\text{-NHCH}_2\text{CO-AA}_1$ -resin

$\text{BrCH}_2\text{CO-AA}_1$ -resin was first washed with DMSO. Sufficient DMSO was added to swell the resin (~12.5 mL/gr of resin) followed by the addition of 40 eq. of the desired primary amine, R^6NH_2 . The reaction was then placed on a spinner and rotated overnight. The reaction was then filtered by suction filtration and washed
10 three times with CH_2Cl_2 , three times with MeOH, once with 1:1 HOAc/ CH_2Cl_2 , three times with MeOH and then lastly three times with CH_2Cl_2 to give $\text{R}^6\text{-NHCH}_2\text{CO-AA}_1$ -resin.

3. $\text{CBz-NR}^6\text{-CH}_2\text{CO-AA}_1$ -resin

15 To $\text{R}^6\text{-NHCH}_2\text{CO-AA}_1$ -resin is added 3 eq. of CBZOSu, 3 eq. Et_3N and 0.05 eq. of a 0.116 M solution of 4-dimethylaminopyridine in THF. Sufficient CH_2Cl_2 was added to swell the resin (~12.5 mL/gr of resin). The reaction was then placed on a spinner and rotated overnight. The reaction was then filtered by suction filtration and washed three times with CH_2Cl_2 , three times with MeOH, once with
20 1:1 HOAc/ CH_2Cl_2 , three times with MeOH and lastly three times with CH_2Cl_2 to give $\text{CBz-NR}^6\text{-CH}_2\text{CO-AA}_1$ -resin.

4. Removal of protecting groups

If AA_1 or AA_2 contains an acid labile side chain protecting group that is to
25 be removed, the resulting resin ($\text{CBz-NR}^6\text{-CH}_2\text{CO-AA}_1$ -resin) was treated with a solution of 95/2.5/2.5 TFA/ H_2O /triisopropyl silane (TIS) for 2 hours. The reaction

was then filtered by suction filtration and washed three times with CH_2Cl_2 , three times with MeOH, and then three times with CH_2Cl_2 .

5. $\text{CBz-NR}^6\text{-CH}_2\text{CO-AA}_1\text{-NHOH}$

5 $\text{CBz-NR}^6\text{-CH}_2\text{CO-AA}_1\text{-resin}$ was first washed with THF. Sufficient THF was added to swell the resin (~ 12.5 mL/gr of resin) and then 25 eq. of 50% aq. NH_2OH was added and the reaction was rotated for two days. The reaction was then filtered by suction filtration and washed with CH_2Cl_2 , MeOH and finally CH_2Cl_2 . The filtrate was concentrated under vacuum to obtain $\text{CBz-NR}^6\text{-CH}_2\text{CO-AA}_1\text{-NHOH}$.
10 NHOH .

Example VII

General experimental for $\text{R}^7\text{NHCO-AA}_2\text{-AA}_1\text{-NHOH}$

(Dipeptide Compounds of Formula I where Z is $-\text{C(O)NH-}$)

15

1. To $\text{ArOCO-AA}_2\text{-AA}_1\text{-resin}$ ($\text{Ar} = \text{Ph}$) was added sufficient DMF to swell the resin (~ 12.5 mL/gr of resin). 20 eq. of R^7NH_2 was added and the reaction reaction was then placed on a spinner and rotated overnight. The reaction was then filtered by suction filtration and washed three times with CH_2Cl_2 , three times with MeOH,
20 once with 1:1 HOAc/ CH_2Cl_2 , three times with MeOH and then lastly three times with CH_2Cl_2 to give $\text{R}^7\text{NHCO-AA}_2\text{-AA}_1\text{-resin}$.

25

2. $\text{R}^7\text{NHCO-AA}_2\text{-AA}_1\text{-resin}$ was first washed with THF. Sufficient THF was added to swell the resin (~ 12.5 mL/gr of resin), then 25 eq. of 50% aq. NH_2OH was added and the reaction was rotated for two days. The reaction was then filtered by suction filtration and washed with CH_2Cl_2 , MeOH, and then CH_2Cl_2 . The filtrate was concentrated under to obtain $\text{R}^7\text{NHCO-AA}_2\text{-AA}_1\text{-NHOH}$.

Example VIIIGeneral experimental for CBz-AA₃-AA₂-AA₁-NHOH

(Tripeptide Compounds of Formula I where n = 1 and ZR⁷ is benzyloxycarbonyl)

5 1. CBz-AA₃-AA₂-AA₁-resin

To H₂N-AA₂-AA₁-resin was added three eq. of CBZ protected AA₃, three eq. of diisopropylcarbodiimide and 0.05 eq. of a 0.116 M solution of dimethylaminopyridine in THF. Sufficient CH₂Cl₂ is added to swell the resin (~12.5 mL/gr of resin). [An alternative is to add to the resin three eq. of CBz protected AA₃, three eq. of HATU, three eq. HOAt and six eq. of DIPEA. Sufficient NMP was added to swell the resin (~12.5 mL/gr of resin).] The reaction was placed on a spinner and rotated overnight. The reaction was filtered by suction filtration and washed three times with CH₂Cl₂, three times with MeOH, once with 1:1 HOAc/CH₂Cl₂, three times with MeOH and lastly three times with CH₂Cl₂, to give CBz-AA₃-AA₂-AA₁-resin.

2. Removal of protecting groups

If AA₁, AA₂ or AA₃ contains an acid labile side chain protecting group that is to be removed, the resulting resin (CBz-AA₃-AA₂-AA₁-resin) was treated with a solution of 95/2.5/2.5 TFA/H₂O, triisopropyl silane (TIS) for 2 hours. The reaction was then filtered by suction filtration and washed three times with CH₂Cl₂, three times with MeOH, and then three times with CH₂Cl₂.

3. CBz-AA₃-AA₂-AA₁-NHOH

CBz-AA₃-AA₂-AA₁-resin was first washed with THF. Sufficient THF was added to swell the resin (~12.5 mL/gr of resin) then 25 eq. of 50% aq. NH₂OH was added and the reaction was rotated for two days. The reaction was filtered by suction filtration and washed with CH₂Cl₂, MeOH, and finally with CH₂Cl₂. The filtrate was concentrated under vacuum to obtain CBz-AA₃-AA₂-AA₁-NHOH.

Example IXGeneral experimental for $R^7Z-AA_3-AA_2-AA_1-NHOH$

(Tripeptide Compounds of Formula I where Z is $-SO_2-$ or $-C(O)NH-$)

5 1. FMOC- $AA_3-AA_2-AA_1$ -resin

To $H_2N-AA_2-AA_1$ -resin was added three eq. of FMOC protected AA_3 , three eq. of diisopropylcarbodiimide and 0.05 eq. of a 0.116 M solution of 4-dimethylaminopyridine in THF. Sufficient CH_2Cl_2 was added to swell the resin (~12.5 mL/gr of resin). [and alternative was to add to the resin three eq. of FMOC protected AA_3 , three eq. of HATU, three eq. HOAt and six eq. of DIPEA. Sufficient NMP was added to swell the resin (~12.5 mL/gr of resin).] The reaction was placed on a spinner and rotated overnight. The reaction was then filtered by suction filtration and washed three times with CH_2Cl_2 , three times with MeOH, once with 1:1 HOAc/ CH_2Cl_2 , three times with MeOH and then lastly three times with CH_2Cl_2 to give FMOC- $AA_3-AA_2-AA_1$ -resin.

2. $H_2N-AA_3-AA_2-AA_1$ -resin

FMOC- $AA_3-AA_2-AA_1$ -resin was first washed with DMF then treated with a solution of 20% piperidine in DMF for 20 min. The reaction was then filtered by suction filtration and washed three times with CH_2Cl_2 , three times with MeOH, once with 1:1 HOAc/ CH_2Cl_2 , three times with MeOH and then lastly three times with CH_2Cl_2 to give $H_2N-AA_3-AA_2-AA_1$ -resin.

3. $R^7Z-AA_3-AA_2-AA_1$ -resin

25 3a. For $Z = SO_2$: $H_2N-AA_3-AA_2-AA_1$ -resin is first washed with aq. dioxane, then 10 eq. of the desired sulfonyl chloride, R^7SO_2Cl , is added followed by sufficient 90% aq. dioxane to swell the resin (~12.5 mL/gr of resin). Then 20 eq. of diisopropylethylamine was added. The reaction was then placed on a spinner and rotated overnight. The reaction was then filtered by suction filtration and washed three times with CH_2Cl_2 , three times with MeOH, once with 1:1 HOAc/ CH_2Cl_2 ,

three times with MeOH and then lastly three times with CH₂Cl₂ to give R⁷SO₂-AA₃-AA₂-AA₁-resin.

3b. For Z = NHCO: H₂N-AA₃-AA₂-AA₁-resin was first washed with THF and then three eq. of the desired isocyanate, R⁷N=C=O was added followed by sufficient THF to swell the resin (~12.5 mL/gr of resin). The reaction was then placed on a spinner and rotated overnight. The reaction was then filtered by suction filtration and washed three times with CH₂Cl₂, three times with MeOH, once with 1:1 HOAc/CH₂Cl₂, three times with MeOH and then lastly three times with CH₂Cl₂ to give R⁷NHCO-AA₃-AA₂-AA₁-resin.

4. R⁷Z-AA₃-AA₂-AA₁-NHOH

R⁷Z-AA₃-AA₂-AA₁-resin is first washed with THF. Sufficient THF is added to swell the resin (~12.5 mL/gr of resin) then 25 eq. of 50% aq. NH₂OH is added and the reaction is rotated for two days. The reaction is then filtered by suction filtration and washed with CH₂Cl₂, MeOH, and then CH₂Cl₂. The filtrate is concentrated on a Speed Vac to obtain R⁷Z-AA₃-AA₂-AA₁-NHOH.

Example X

General experimental for R⁷Z-NR¹⁰C(O)CH₂-AA₂-AA₁-NHOH

(Tripeptide Compounds of Formula I where n = 1, m = 0, R⁹ is hydrogen (A is C(O)CH₂NR¹⁰) and Z is a bond, -SO₂-, -C=O, or -C(O)NH-)

1. BrCH₂CO-AA₂-AA₁-resin

To H₂N-AA₂-AA₁-resin was added twelve eq. of bromoacetic acid and twelve eq. of diisopropylcarbodiimide. Sufficient CH₂Cl₂ was added to swell the resin (~12.5 mL/gr of resin). The reaction was then placed on a spinner and rotated two hrs. The reaction was then filtered by suction filtration and washed three times with CH₂Cl₂, three times with DMSO and then lastly three times with CH₂Cl₂ to give BrCH₂CO-AA₂-AA₁-resin.

2. $R^{10}NHCH_2CO-AA_2-AA_1$ -resin

BrCH₂CO-AA₂-AA₁-resin is first washed with DMSO. Sufficient DMSO is added to swell the resin (~12.5 mL/gr of resin) followed by the addition of 40 eq. of the desired amine, R¹⁰NH₂ or R⁷R¹⁰NH. The reaction was then placed on a spinner and rotated overnight. The reaction was then filtered by suction filtration and three times with CH₂Cl₂, three times with MeOH, once with 1:1 HOAc/CH₂Cl₂, three times with MeOH and then lastly three times with CH₂Cl₂ to give R¹⁰NHCH₂CO-AA₂-AA₁-resin or R⁷R¹⁰NHCH₂CO-AA₂-AA₁-resin. (If R⁷R¹⁰NH is used, corresponding to Z being a bond, go directly to step 4)

3. $R^7Z-NR^{10}CH_2CO-AA_2-AA_1$ -resin

3a. For Z = -SO₂-. R¹⁰NHCH₂CO-AA₂-AA₁-resin is first washed with aq. dioxane, then 10 eq. of the desired sulfonyl chloride, R⁷SO₂Cl, is added. Sufficient 90% aq. dioxane was added to swell the resin (~12.5 mL/gr of resin). Then 20 eq. of diisopropylethylamine was added. The reaction was then placed on a spinner and rotated overnight. The reaction was then filtered by suction filtration and washed three times with CH₂Cl₂, three times with MeOH, once with 1:1 HOAc/CH₂Cl₂, 3 times with MeOH and then lastly three times with CH₂Cl₂ to give R⁷SO₂-NR¹⁰CH₂CO-AA₂-AA₁-resin

3b. For Z = -CO-. To R¹⁰NHCH₂CO-AA₂-AA₁-resin is added sufficient CH₂Cl₂ is added to swell the resin (~12.5 mL/gr of resin). Three eq. of the desired acid chloride, R⁷COCl, and three eq. of Et₃N are then added. [A first alternative is to add to the resin sufficient CH₂Cl₂ is added to swell the resin (~12.5 mL/gr of resin) followed by three eq. of the desired carboxylic acid, R⁷COOH, 3 eq. of diisopropylcarbodiimide and 0.05 eq. of a 0.116 M solution of dimethylaminopyridine in THF. A second alternative is to add to the resin 3 eq. of the desired carboxylic acid, R⁷COOH, 3 eq. of HATU, 3 eq. HOAt and 6 eq. of DIPEA followed by sufficient NMP is added to swell the resin (~12.5 mL/gr of resin).] The reaction was then placed on a spinner and rotated overnight. The reaction was then filtered by suction filtration and washed three times with CH₂Cl₂, three

times with MeOH, once with 1:1 HOAc/CH₂Cl₂, three times with MeOH and then lastly three times with CH₂Cl₂ to give R⁷CO-NR¹⁰CH₂-AA₂-AA₁-resin

3c. For Z = -CONH-: R¹⁰NHCH₂CO-AA₂-AA₁-resin is first washed with THF, then 3 eq. of the desired isocyanate, R⁷N=C=O, was added followed by sufficient THF to swell the resin (~12.5 mL/gr of resin). The reaction was then placed on a spinner and rotated overnight. The reaction was then filtered by suction filtration and washed three times with CH₂Cl₂, three times with MeOH, once with 1:1 HOAc/CH₂Cl₂, three times with MeOH and then lastly three times with CH₂Cl₂ to give R⁷NHCO-NR¹⁰CH₂-AA₂-AA₁-resin.

4. Removal of protecting groups

If AA₁ or AA₂ contains an acid labile side chain protecting group that is to be removed, the resulting resin (R⁷Z-NR¹⁰CH₂CO-AA₂-AA₁-resin) was treated with a solution of 95/2.5/2.5 TFA/H₂O, triisopropyl silane (TIS) for 2 hours. The reaction was then filtered by suction filtration and washed three times with CH₂Cl₂, three times with MeOH, and then three times with CH₂Cl₂.

5. R⁷Z-NR¹⁰C(O)CH₂-AA₂-AA₁-NHOH

R⁷Z-NR¹⁰CH₂CO-AA₂-AA₁-resin was first washed with THF. Sufficient THF was added to swell the resin (~12.5 mL/gr of resin) then 25 eq. of 50% aq. NH₂OH was added and the reaction was rotated for two days. The reaction was then filtered by suction filtration and washed with CH₂Cl₂, MeOH, and then CH₂Cl₂. The filtrate was concentrated under vacuum to obtain R⁷Z-NR¹⁰C(O)CH₂-AA₂-AA₁-NHOH

*Note in all cases, all solid reagents are added followed by solvent. If the reagents are not solids, the solvent is added followed by the liquid reagents.

For AA₁ or AA₂ with side chain that require deprotection this was done just prior to the NH₂OH cleavage reaction. Typical amino acids with side chains requiring deprotection include Glu (OtBu), His (Boc) and Ser (OtBu).

Example XI

Isolation and Preparation of Procollagen C-Proteinase

Cloning of Human PCP and Construction of the HT-1080 Vector

5

Human Procollagen C-Proteinase (PCP, also known as Bone Morphogenetic Protein-1 or BMP-1) was cloned by from a human fibroblast cDNA library (Stratagene, San Diego, CA). Cloning was performed by PCR based on the reported nucleotide sequence (Wozney, J.M., Rosen, V., Celeste, A.J., Mitsock, L.M., Whitters, M.J., Kriz, R.W., Hewick, R.M., and Wang, E.A. (1989) direct GenBank submission accession M22488, locus HUMBMP1) using Taq polymerase, the 5' primer

10

15

GCGCGCGGTACCCGCCCCGCCAGCATGCCCGGCGTGGCCCGCCTGCCGC
TGCTGCTCGGGCTGCTGCTGCTCCCGCGTCCCGGCCGGCCGCTGGACTTG
GCCGACTACACCTATGACCTGGC (SEQ ID NO:1)(Oligo Therapeutics, Inc.,
Wilsonville, OR), and the 3' reverse strand primer

20

25

30

CCGCTCGAGCCTCACTGGGGGGTCCGGTTTCTTTTCTGCACTCGGAATTT
GAGCTGGTG (SEQ ID NO:2) (Gibco) to yield the entire full-length nucleotide encoding the signal sequence, propeptide, catalytic domain, and all C-terminal domains to the natural translation termination site. The PCR product was purified by gel electrophoresis using the Wizard DNA Purification Kit (Promega, Madison, WI) and ligated directly into the mammalian expression vector pCR3.1 (Invitrogen, Carlsbad, CA) by the TA cloning method. Ligated product was used to transform E coli strain TOP10F' (Invitrogen, Carlsbad, CA) by a standard heat-shock method, and transformants were selected by restriction analysis of purified plasmid using the enzymes HindIII and BamHI. Transformants testing positive for the PCP insert were submitted for sequencing using the Perkin-Elmer / ABI system. Two clones were selected that, combined, encoded the entire amino acid sequence identical to the one predicted by Wozney et al. The two clones were recombined by restriction using the enzymes BbrI, which cleaved at a naturally occurring internal site, and EcoRV,

which cleaved at the junction of the insert and vector. The excised fragments were religated into EcoRV-treated pCR3.1. The resulting construct contained the entire coding sequence identical to that reported by Wozney et al. with the exception of two silent mutations in the signal sequence, G->A at both positions 39 and 45 counting from the translation initiation site (ATG). The completed plasmid construct was amplified in *E. coli* DH5 α and purified using anion exchange chromatography (MaxiPrep columns from Qiagen (Valencia, CA) catalog #12162).

Transfection of HT-1080 and Selection of the PCP-Expressing Clone

The human fibrosarcoma line HT-1080 (ATCC) was grown in high glucose DMEM (DMEM-HG) supplemented with 10 % heat-inactivated fetal bovine serum (HI-FBS) in 100 mm culture dishes (Falcon, Becton Dickinson, Franklin, NJ) and transfected with 2 μ g of purified plasmid using the standard method for Lipofectamine (Gibco, Bethesda, MD) in serum free medium. Stable transfectants were selected by treating the plated culture with 400 μ g/ml G418 (Gibco). After selection for 10 days, the adherent single colonies were picked from the plate, replated in 12-well plates and grown until confluent. Individual stable colonies were screened for PCP expression by TaqMan (Perkin-Elmer, Foster City, CA) analysis using equivalent amounts of total RNA, the 5' primer GACGAAGAGGACCTGAGGGCCTT (SEQ ID NO:3) (Perkin-Elmer, Foster City, CA), the 3' reverse strand primer TTCCTGGAAGTGCAGCTTTGA (SEQ ID NO:4) (Perkin-Elmer, Foster City, CA), and the reverse strand probe TGCCGTCTGAGATCCACAGCCTGCT (SEQ ID NO:5) (Perkin-Elmer). A stable line, HT-1080/hPCP-23, was chosen based on the highest PCP mRNA expression level in the TaqMan screen. Stocks of the HT-1080/hPCP-23 stable line were transferred to DMEM-HG supplemented with 5 % HI-FBS and 10 % DMSO (no G418 added) and were slowly frozen at -70 °C overnight, then transferred to a liquid nitrogen bath for long-term storage. Revitalized HT-1080/hPCP-23 were maintained in DMEM-HG supplemented with 10 % HI-FBS and 250 μ g/ml G418 for no more than 7 passages. Expression of PCP for harvest was carried out by replating and

growing HT-1080/hPCP-23 on rat tail type I collagen-coated plates (Falcon) in OptiMEM (Gibco) serum free medium without G418 for 24 hr.

5 Production of PCP in HT1080 cells

The HT1080 cells that were transformed to produce PCP were adapted to grow in suspension in optiMEM medium (GIBCO) supplemented with 5% fetal bovine serum and 4 ml/L G418 (GIBCO). The culture was maintained at 37C and the dissolved oxygen at 30%. Typically batch sizes of 10 liters were produced.

10 When the cell density reached $4-6 \times 10^5$ cells/ml, the culture fluid was collected and filtered through 0.2 um membranes. Alternatively, the cell culture was perfused with fresh media at the rate of 0.8 to 1.0 culture volume/day. The density of the perfused cultures reached $1-2.5 \times 10^6$ cells/ml and were maintained up to two weeks with continuous harvests.

15

Purification of PCP from HT1080 Cells

A column packed with Dyematrix Gel Green A (Millipore, Bedford, MA) was equilibrated against 50 mM HEPES, pH7.2, containing 6mM CaCl_2 and 0.3M NaCl. After the HT1080 cell culture fluid was loaded, the column was washed with

20 10 column volumes of the equilibration buffer containing 1.0 M NaCl. PCP was eluted with 50 mM HEPES pH 7.2 containing 3 M NaCl, 2 M urea and 6 mM CaCl_2 . Eluate fractions were pooled and concentrated to 150-200 mls and dialyzed against 4.0 liters of 50 mM HEPES, 6 mM CaCl_2 , pH 7.2 overnight. The material was then centrifuged at 5,000 g for 15 minutes to remove precipitates. The PCP containing

25 sample were stored at -20C until ready for further processing.

The PCP containing sample was thawed and diluted with 50 mM HEPES pH 7.2 containing 6 mM CaCl_2 , if necessary to bring the NaCl concentration to 0.1-0.15 M. The pH was adjusted to 6.7 with 2 N HCl. The protein solution was filtered

30 through a 0.45 um filter to remove any precipitate. This preparation was then loaded

onto a column packed with Q-Sepharose High Performance (Pharmacia, Piscataway, NJ) which had been equilibrated with 50mM HEPES pH 6.7 containing 6 mM CaCl₂ and 0.15 M NaCl. The PCP was not retained in the column and was therefore in the flow through fractions. The PCP was concentrated to 1 mg/ml and used for screening.

Production of PCP in Drosophila Cells

Drosophila cells which had been transformed to produce PCP were grown in bioreactors at a typical batch volume of 10 liters in SF900 II SF medium (GIBCO). The temperature was maintained at 30C and the dissolved oxygen at 30%. Periodically the cells were fed a cocktail consisting of glutamine, lipids and yeastolate. When cell densities reached 30-50 X 10⁶ cells/ml, supernatants were harvested by centrifugation and concentrated by ultrafiltration using a 30Kd membrane.

Purification of PCP from Culture Fluid from Drosophila Cells

Culture fluid from the drosophila cells was concentrated 8 fold and the pH adjusted to 7.1-7.2 if necessary. The culture fluid was centrifuged at 3000 g for 10 minutes and filtered through 0.45 um filters. The culture fluid was then loaded onto columns packed with carboxy-sulfone packing material (J.T. Baker/Mallinckrodt, Phillipsburg, NJ) which had been equilibrated with 0.1 M NaCl, 50 mM HEPES, 6 mM CaCl₂, pH 7.2. After being loaded, the column was washed with 10 column volumes of the equilibration buffer. Retained proteins were eluted with a gradient of 0.1 to 1.0 M NaCl in 9 column volumes. Fractions that had PCP activity were pooled for further purification.

The PCP eluted off the carboxy-sulfone column was loaded onto a Dyematrix Gel Green A (Millipore, Bedford, MA) column that had been equilibrated with 50 mM HEPES, pH 7.4 containing 0.3 M NaCl and 6 mM CaCl₂. The column was then washed with the equilibration buffer containing 1 M NaCl.

Retained proteins were eluted with 50 mM HEPES, pH 7.4, 3 M NaCl, 2 M urea, 6 mM CaCl₂. The elution peak was concentrated and dialyzed against 50 mM HEPES, pH 7.4 containing 0.3 M NaCl, 6 mM CaCl₂. The preparation was centrifuged at 3000 g for 10 minutes. Brij 35 (Sigma, Madison, WI) was added to the supernatant to a final concentration of 0.02%. This preparation was used for screening.

Example XII

Isolation of Collagenase enzymes

The catalytic domain of human collagenase-1 was expressed as a fusion protein with ubiquitin in *E. Coli* as described in Gehring, E.R. *et al.*, *J. Biol. Chem.*, 270, 22507, (1995). After purification of the fusion protein, the collagenase-1 catalytic domain was released by treatment with 1mM of aminophenylmercuric acetate (APMA) for 1 hour at 37 °C and then purified by zinc chelate chromatography.

Human collagenase-2 and gelatinase B were isolated in active form from buffy coats as described in Mookhtiar, K.A. *et al.*, *Biochemistry*, 29, 10620, (1990).

The propeptide and catalytic domain portion of human collagenase-3 was expressed in *E. Coli* as an *N*-terminal fusion protein with ubiquitin. After purification, the catalytic domain was released by treatment with 1 mM APMA for 1 hour at 37 °C, and then purified by zinc chelate chromatography.

Rat collagenase-3 was purified in active form from the culture media of uterine smooth muscle cells as described in Roswit, W.T. *et al.*, *Arch. Biochem. Biophys.*, 225, 285-295 (1983).

Example XIII

Inhibition of Procollagen C-Proteinase activity

The ability of the compounds to inhibit PCP has been demonstrated in the following *in vitro* assays utilizing a synthetic peptide as the substrate.

Assay A

5 A continuous assay was performed using 20 μ M substrate (Dabcyl-Pro-Tyr-Tyr-Gly-Asp-Glu-Pro-n-Leu-Edans) (SEQ ID NO:6). The final assay conditions were 20 μ M substrate, 50 mM HEPES pH 7.5, 50 mM NaCl, 3% DMSO, 37°C and PCP enzyme. Product formation was monitored by fluorescence spectroscopy, Ex. = 335 nm, Em. = 490 nm. The IC₅₀ was calculated from plots of the initial velocity vs.
10 compound concentration.

Assay B

 Eighty μ L of buffer A (20 mM HEPES) containing the desired concentrations of the test compound in DMSO or carrier vehicle was mixed with 10 μ L of approx.
15 1mg/mL PCP enzyme and 10 μ L of 0.1mM substrate both in 20mM HEPES. The contents are mixed, incubated at room temperature for 1-2 hours and fluorescent readings taken with a Victor plate reader (Ex. 405nm, Em. 460nm at 2000-40,000 lamp energy, 0.1-1 sec/well). The substrate was DACM-Cys-Pro-Tyr-Gly-Asp-Glu-Pro-nLeu-Lys-FITC-OH. (SEQ ID NO:7) (DACM = dimethylamino-coumarylmaleimide, FITC = fluorescein isothiocyanate). The IC₅₀ was calculated
20 from plots of the initial velocity vs. compound concentration.

 Additional *in vitro* assays using native procollagen as the substrate may also be used and these assays are described in more detail in WO 97/05865 ("C-Proteinase
25 Inhibitors for the Treatment of Disorders Relating to the Overproduction of Collagen").

 The compounds in Tables I-III, V and VI had IC₅₀'s in the range of 0.02 to 200 μ M.

The compounds in Table IV had IC₅₀'s in the range of 10-1000 µM.

Example XIV

Measurement of Collagenase Activity

5 The collagenase-1, collagenase-2 and collagenase-3 inhibitory activity of compounds of this invention *in vitro* was determined based on the hydrolysis of MCA-Pro-Leu-Gly-Leu-DPA-Ala-Arg-NH₂ (SEQ ID NO:8) (Bachem, Inc.) at 37 °C as described in Knight, C.G., *et al.*, FEBS Lett., 296(3): 263-266 (1992).

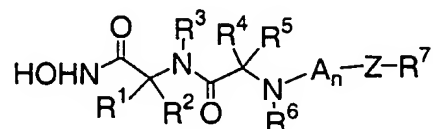
10 The collagenase enzyme was diluted with assay buffer (50 mM Tricine pH 7.5, 200 mM NaCl, 10 mM CaCl₂, and 0.005% Brij-35) containing 10 µmole of above substrate dissolved in DMSO. Compounds of the invention dissolved in DMSO or only DMSO (control samples) were added such that the final DMSO concentration in all assays was 2.5%. The fluorescence changes were monitored with
15 a Perkin-Elmer LS-50B fluorimeter using an excitation wavelength of 328 nm and an emission wavelength of 393 nm.

 Selected compounds from Tables I-VI were 10-1000 more selective for PCP inhibition than for the human collagenase enzymes.

Claims

1. A compound selected from the group of compounds represented by Formula (I):

5



(I)

wherein:

R^1 and R^4 are, independently of each other, hydrogen or alkyl;

- R^2 is: (i) cycloalkyl, cycloalkylalkyl, aryl, aralkyl, aralkenyl, heteroaryl, heteroaralkyl, heteroaralkenyl, heterocyclo or heterocycloalkyl; or
 10 (ii) -(alkylene)-B-X where B is -O-, -NR⁸-, -S(O)_n- (where n is 0, 1 or 2), -C=O, -CONR⁸-, -NR⁸CO₂-, NR⁸SO₂- or -C(=NR⁸)NR⁸SO₂- (where R⁸ is H or alkyl), and X is cycloalkyl, cycloalkylalkyl, aryl, aralkyl heteroaryl or heteroaralkyl; or

- 15 (iii) -(alkylene)-B-X where B is -NR⁸CO- (where R⁸ is H or alkyl), and X is cycloalkyl, cycloalkylalkyl, aryl, aralkyl, heteroaryl or heteroaralkyl; or

(iv) R^2 and R^3 form an alkylene or heteroalkylene chain;

R^3 is hydrogen or alkyl;

- R^6 is hydrogen, alkyl, cycloalkyl, cycloalkylalkyl, aryl, aralkyl, heteroaryl or
 20 heteroaralkyl;

R^5 is:

- (i) hydrogen, alkyl, cycloalkyl, cycloalkylalkyl, aryl, aralkyl, aralkenyl, heteroaryl, heteroaralkyl, heteroaralkenyl, heterocycloalkyl, heteroalkyl, or
 -(alkylene)-C(O)-X¹ where X¹ is alkyl, hydroxy, alkoxy, aryl, aralkyl, aryloxy, aralkyloxy, heteroaryl, heteroaryloxy, heteroaralkyloxy or NR'R'' (where R' and R''
 25 are independently H or alkyl, or R' and R'' form an alkylene chain); or

(ii) R^5 and R^4 form an alkylene chain; or

(iii) R^5 and R^6 form an alkylene chain;

n is 0 or 1;

A is $-C(=O)-CH(R^9)-(CH_2)_m-N(R^{10})-$ wherein:

5 m is an integer from 0-5 inclusive;

R^9 is hydrogen, alkyl, cycloalkyl, cycloalkylalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl, heterocycloalkyl, heteroalkyl, or $-(alkylene)-C(O)-X^1$ where X^1 is alkyl, hydroxy, alkoxy, aryl, aralkyl, aryloxy, aralkyloxy, heteroaryl, heteroaryloxy, heteroaralkyloxy or $NR'R''$ (where R' and R'' are independently H or alkyl, or R' and R'' form an alkylene chain); and

10 R^{10} is hydrogen, alkyl, aralkyl or heteroaralkyl;

Z is Y-B wherein:

Y is alkylene or a bond; and

B is $-CO-$, $-C(O)O-$, $-CONR^8-$, $-SO_2-$, or $-SO_2NR^8-$ (where R^8 is hydrogen or alkyl), alkylene (optionally substituted by hydroxy, alkoxy, amino, monoalkylamino or dialkylamino) or a bond;

R^7 is cycloalkyl, cycloalkylalkyl, aryl, aralkyl, heteroaryl or heteroaralkyl;

provided that when $n = 0$ and Z is SO_2 , then R^2 does not contain an imidazole group; and their pharmaceutically acceptable salts, prodrugs, individual isomers, and mixtures of isomers.

20

2. The compound of Claim 1 wherein:

n is 0.

25 3. The compound of Claim 2 wherein R^3 and R^6 are hydrogen.

4. The compound of Claim 3, wherein:

R^2 is aralkyl or heteroaralkyl.

5. The compound of Claim 4 wherein:
Z is -C(O)O- or -S(O)₂-.
6. The compound of Claim 5 wherein:
5 R² is optionally substituted benzyl or heteroaralkyl.
7. The compound of Claim 6 wherein, R² is 4-t-butoxybenzyl, 3-chlorobenzyl, 3-indolyl methyl, 2-thienylmethyl, 4-imidazolylmethyl or 4-thiazolylmethyl.
- 10 8. The compound of Claim 7 wherein R² is 4-thiazolylmethyl.
9. The compound of Claim 8 wherein:
R⁷ is aryl, aralkyl, heteroaryl or heteroaralkyl.
- 15 10. The compound of Claim 8 wherein:
Z is -C(O)O- and R⁷ is optionally substituted benzyl.
11. The compound of Claim 9 wherein:
Z is -SO₂- and R⁷ is aryl or heteroaryl.
- 20 12. The compound of Claim 10 or Claim 11, wherein:
R¹ and R⁴ are hydrogen and R⁵ is alkyl.
13. The compound of Claim 12 wherein R⁵ is (S,S)-1-methylpropyl.
- 25 14. The compound of Claim 3, wherein:
R² is (alkylene)-B-X where B is -O-, -NR⁸-, -S(O)_n- (where n is 0, 1 or 2), -C=O, -CONR⁸-, -NR⁸CO₂-, -NR⁸SO₂- or -C(=NR⁸)NSO₂- (where R⁸ is H or alkyl), and X is cycloalkyl, cycloalkylalkyl, aryl, aralkyl, heteroaryl or heteroaralkyl.

15. The compound of Claim 14, wherein:
Z is -C(O)O- or -S(O)₂-.
16. The compound of Claim 15, wherein R² is -CH₂-B-X and
5 B is -NHCO₂- and X is benzyl.
17. The compound of Claim 16 wherein:
R⁷ is aryl or aralkyl.
- 10 18. The compound of Claim 17, wherein:
R¹ and R⁴ are hydrogen and R⁵ is alkyl.
19. The compound of Claim 18 wherein R⁵ is (S,S)-1-methylpropyl.
- 15 20. The compound of Claim 1 wherein:
n is 1.
21. The compound of Claim 20 wherein m is 0 and R³ and R⁶ are hydrogen.
- 20 22. The compound of Claim 21, wherein:
R² is aralkyl or heteroaralkyl.
23. The compound of Claim 22, wherein:
Z is -C(O)O- or -S(O)₂-.
- 25 24. The compound of Claim 23, wherein:
R² is optionally substituted benzyl or heteroaralkyl.
25. The compound of Claim 24 wherein R² is 4-t-butoxybenzyl, 3-chlorobenzyl,
30 3-indolyl methyl, 2-thienylmethyl, 4-imidazolylmethyl or 4-thiazolylmethyl.

26. The compound of Claim 25 wherein R^2 is 4-thiazolylmethyl.
27. The compound of Claim 26 wherein:
5 R^7 is aryl, aralkyl, heteroaryl or heteroaralkyl.
28. The compound of Claim 27 wherein:
 Z is $-C(O)O-$ and R^7 is benzyl.
- 10 29. The compound of Claim 27 wherein:
 Z is $-SO_2-$ and R^7 is aryl.
30. The compound of Claim 28 or 29, wherein:
 R^1 and R^4 are hydrogen and R^5 is alkyl.
- 15 31. The compound of Claim 30 wherein R^5 is (S,S)-1-methylpropyl.
32. The compound of Claim 21, wherein:
 R^2 is (alkylene)-B-X where B is $-O-$, $-NR^8-$, $-S-$, $-C=O$, $-CONR^8-$, $-NR^8CO_2-$, $-$
20 NSO_2- or $-C(=NR^8)NSO_2-$ (where R^8 is H or alkyl), and X is cycloalkyl, cycloalkylalkyl,
aryl, aralkyl heteroaryl or heteroaralkyl.
33. The compound of Claim 32, wherein:
 Z is $-C(O)O-$ or $-S(O)_2-$.
- 25 34. The compound of Claim 33, wherein R^2 is CH_2-B-X and
B is $-NHCO_2-$ and X is benzyl.
35. The compound of Claim 34 wherein:
30 R^7 is aryl or aralkyl.

36. The compound of Claim 35, wherein:

R^1 and R^4 are hydrogen and R^5 is alkyl.

5 37. The compound of Claim 36 wherein R^5 is (S,S)-1-methylpropyl.

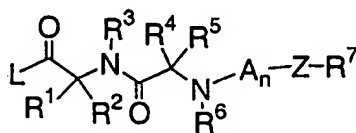
38. A pharmaceutical composition comprising the compound of any one of Claims 1 to 37 and a pharmaceutically acceptable excipient.

10 39. A process for preparing the compounds of Claim 1 by:

(i) treating a compound of Formula II wherein L is a leaving group and $R^1 - R^7$, A, n and Z are as defined in Claim 1 with hydroxylamine or a protected derivative thereof, and

(ii) deprotecting as necessary and isolating the compound of Claim 1.

15



(II)

40. A compound as claimed in Claim 1 whenever prepared according to a process as claimed in Claim 39.

20

41. A compound as claimed in any one of Claims 1 to 37 as a therapeutically active substance.

25

42. Use of a compound as claimed in any one of Claims 1 to 37 for the preparation of a medicament comprising a compound as active ingredient as claimed in any one of Claims 1 to 37 for the treatment of a fibrotic disease, preferably acute respiratory distress syndrome.

43. A method of treating fibrosis comprising administering to a patient in need thereof an inhibitor of procollagen C-proteinase that is at least ten-fold more selective for procollagen C-proteinase over both collagenase-1, collagenase-2 and collagenase-3.

5

44. The invention as hereinbefore described.

SEQUENCE LISTING

5 <110> Dankwardt, Sharon Marie
 Van Wart, Harold
 Walker, Keith Adrian Murray

10 <120> Peptidic Procollagen C-proteinase
 Inhibitors

 <130> 20425

 <160> 8

15 <170> FastSEQ for Windows Version 3.0

 <210> 1
 <211> 122
 <212> DNA

20 <213> Artificial Sequence

 <220>
 <223> pcr primer

25 <400> 1
 gcgcgcggta cccgccccgc cagcatgcc gccgtggccc gcctgccgct gctgctcggg
 60
 ctgctgctgc tcccgcgtcc cggccggccg ctggacttgg ccgactacac ctatgacctg
 120
30 gc
 122

<210> 2
<211> 59
<212> DNA
<213> Artificial Sequence

5

<220>
<223> pcr primer

<400> 2

10 ccgctcgagc ctactgggg ggtccggttt cttttctgca ctcggaattt gagctggtg
59

<210> 3
<211> 23
<212> DNA
<213> Artificial Sequence

15

<220>
<223> pcr primer

20

<400> 3
gacgaagagg acctgagggc ctt
23

25

<210> 4
<211> 21
<212> DNA
<213> Artificial Sequence

30

<220>
<223> pcr primer

<400> 4
ttcctggaac tgcagctttg a
35 21

5 <210> 5
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> pcr primer
10 <400> 5
tgccgtctga gatccacagc ctgct
25

15 <210> 6
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
20 <223> synthetic peptide

<400> 6
Pro Tyr Tyr Gly Asp Glu Pro Leu
1 5
25

<210> 7
<211> 9
<212> PRT
<213> Artificial Sequence

30 <220>
<223> synthetic peptide

<400> 7

Cys Pro Tyr Gly Asp Glu Pro Leu Lys

1

5

5

<210> 8

<211> 7

<212> PRT

<213> Artificial Sequence

10

<220>

<223> synthetic peptide

<223> Xaa = DPA

15

<400> 8

Arg Ala Xaa Leu Gly Leu Pro

1

5

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 99/09519

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07K5/06 C07C259/06 C07K5/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07K C07C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	JP 09 025293 A (TEIJIN LTD) 28 January 1997 (1997-01-28) whole document, esp. Table on page 893	1-44
X	JP 01 146896 A (FUJI YAKUHI KOGYO KK) 8 June 1989 (1989-06-08)	1-3, 14, 20, 38-41, 43
Y	whole document, esp. Table on page 27	1-44
Y	WO 95 12612 A (WARNER LAMBERT CO) 11 May 1995 (1995-05-11) claims, examples	1-44
Y	WO 92 22523 A (RES CORP TECHNOLOGIES INC) 23 December 1992 (1992-12-23) claims, examples	1-44
	-/-	



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents:

A document defining the general state of the art which is not considered to be of particular relevance

E earlier document but published on or after the international filing date

L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

O document referring to an oral disclosure, use, exhibition or other means

P document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

Z document member of the same patent family

Date of the actual completion of the international search

5 April 2000

Date of mailing of the international search report

14/04/2000

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax (+31-70) 340-3016

Authorized officer

Kronester-Frei, A

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 99/09519

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 96 33176 A (DU PONT MERCK PHARMA) 24 October 1996 (1996-10-24) Table 1, page 54 ff Claims, examples, -----	1-44
P,Y	WO 98 55449 A (QUEENSLAND INST MED RES ;FAIRLIE DAVID (AU); PARSONS PETER G (AU);) 10 December 1998 (1998-12-10) claims, examples, esp. page 46, line 12 and page 5, lines 28/29 -----	1-44

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 99/09519

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
JP 09025293 A	28-01-1997	NONE	
JP 01146896 A	08-06-1989	NONE	
WO 9512612 A	11-05-1995	AU 681454 B AU 7976094 A CA 2170766 A EP 0730605 A FI 961819 A HU 75308 A JP 9504547 T NO 961814 A NZ 275691 A US 5830868 A	28-08-1997 23-05-1995 11-05-1995 11-09-1996 29-04-1996 28-05-1997 06-05-1997 06-05-1996 25-03-1998 03-11-1998
WO 9222523 A	23-12-1992	AU 2228292 A US 5616605 A US 5387610 A	12-01-1993 01-04-1997 07-02-1995
WO 9633176 A	24-10-1996	US 5703092 A AU 5665396 A CA 2218345 A EP 0821675 A JP 11504015 T	30-12-1997 07-11-1996 24-10-1996 04-02-1998 06-04-1999
WO 9855449 A	10-12-1998	AU 7751698 A EP 0988280 A	21-12-1998 29-03-2000